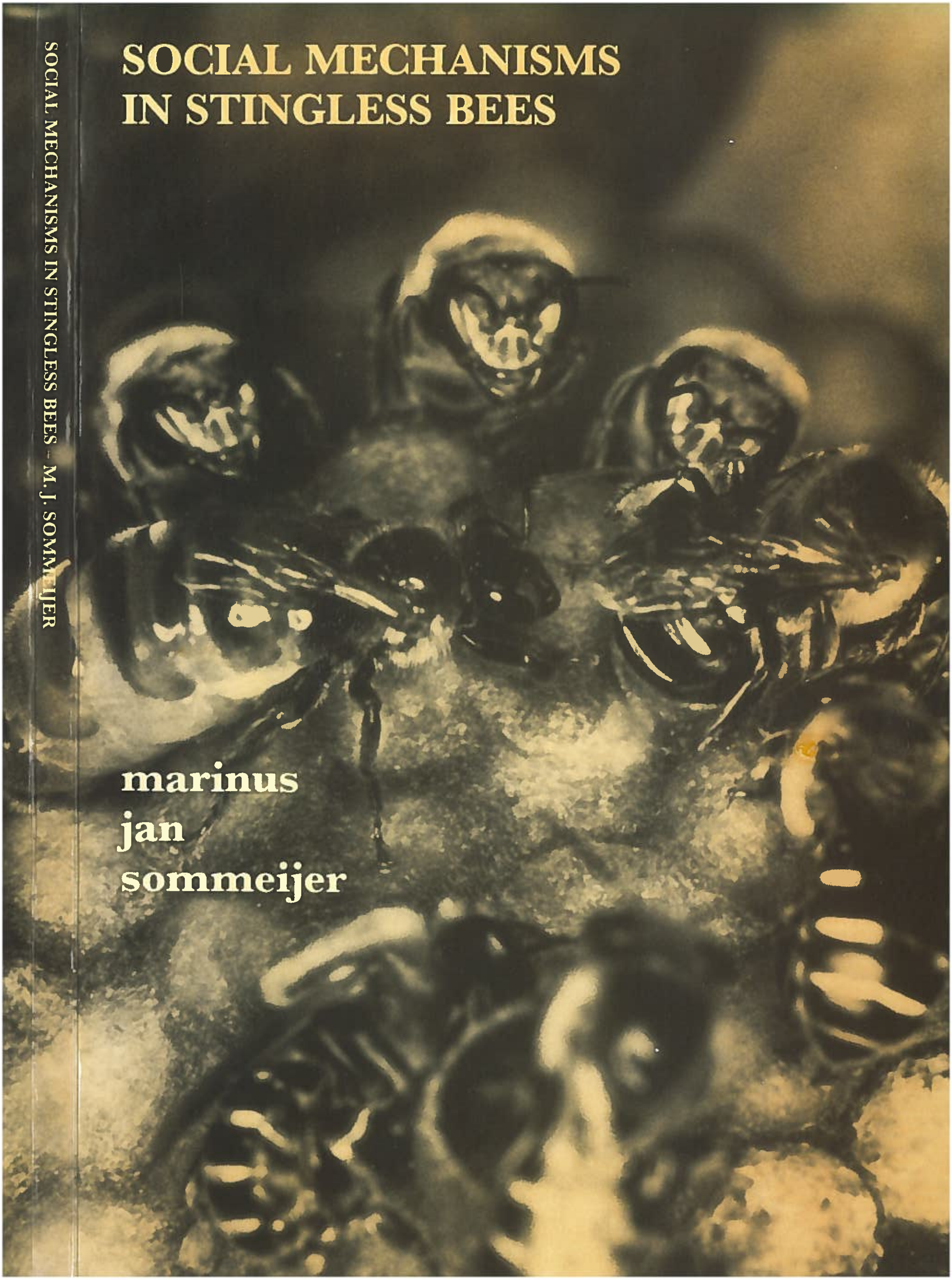
SOCIAL MECHANISMS IN STINGLESS BEES



**marinus
jan
sommeijer**

SOCIAL MECHANISMS IN STINGLESS BEES – M.J. SOMMEIJER

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN
DE WISKUNDE EN NATUURWETENSCHAPPEN AAN DE
RIJKSUNIVERSITEIT TE UTRECHT, OP GEZAG VAN DE
RECTOR MAGNIFICUS PROF. DR. O.J. DE JONG, VOLGENS BESLUIT VAN HET COLLEGE VAN DECANEN IN
HET OPENBAAR TE VERDEDIGEN OP WOENSDAG
7 DECEMBER 1983 DES NAMIDDAGS TE 4.15 UUR

DOOR

MARINUS JAN SOMMEIJER

geboren op 28 november 1944 te Ovezande

Krips Repro Meppel

PROMOTOR : PROF. DR. J.A.R.A.M. VAN HOOFF

COREFERENTEN : DR. H.H.W. VELTHUIS
PROF. DR. S.F. SAKAGAMI,
Hokkaido University, Japan

Dit proefschrift kwam tot stand mede onder leiding van Dr. H.H. W. Velthuis

STELLINGEN

I De dominatiehiërarchie die Cole aantrof onder de werksters van Leptothorax allardycei is onder de eusociale insekten geen exclusiviteit.

B.J. Cole, 1981. Science, 212: 83-84.

R.M. May, 1981. Nature, 291: 281.

II Het bestaan van een "sociale maag" als systeem waarbij vloeibaar voedsel in alle richtingen onwillekeurig tussen alle leden van de sociale insektenkolonie wordt doorgegeven, moet sterk worden betwijfeld.

III Het opmerkelijke gedrag waardoor bij veel angelloze bijen het beleggen van een aantal broedcellen in de tijd is gekoncentreerd, kan beschouwd worden als een aanpassing ter bevordering van de reproduktieve dominantie van de koningin.

IV De aanzienlijke produktie van mannetjes door werksters van Melipona, die door Silva kon worden aangetoond door de eigen koningin van een kolonie te vervangen door een andersoortige, kan op grond van de kennis over de invloed van de aanwezigheid van de koningin op de oögenese van de werksters als verschijnsel van moerloosheid worden geinterpreteerd.

V De argumenten die Thompson in zijn beschouwing over het begrip adaptatie aanvoert om aan te tonen dat eusocialiteit bij insekten niet als voorbeeld van adaptatie mag gelden, zijn niet overtuigend.

N.S. Thompson, 1981. Perspectives in Ethology 4: 51-73.

VI De samenstelling van de honing van angelloze bijen waaraan in veel Centraal- en Zuidamerikaanse gebieden bizondere geneeskrachtige werking wordt toegeschreven en die gekenmerkt is door houdbaarheid bij een hoog watergehalte moet nodig onderzocht worden.

K. van Deursen, 1981. Surinaamse Imkerkoerier 31: 1-4; 32: 2-5.

M. Gonnet, P. Lavie & P. Nogueira-Neto, 1964. C.R. Acad. Sc. Paris 258: 3107-3109.

D. Roubik, 1983. J. Kans. Entomol. Soc. 56 (3): 327-355.

VII Een duidelijke beperking van de duur van een op landbouwkundig onderzoek gericht ontwikkelingsprojekt helpt te voorkomen dat dit projekt als een permanente of als een afgeronde technische service beschouwd gaat worden en vermindert tevens bij nationale specialisten de vrees dat vooral de buitenlandse ontwikkelingswerkers de wetenschappelijke vruchten zullen plukken van lokale mogelijkheden.

VIII Het propageren van hybride maisvarieteiten voor de kleinschalige landbouw in Centraal-Amerika moet sterk worden afgeraden.

IX Een enge, in de zin van beperkte, sociobiologie bestaat niet.

G. Huis in 't Veld. Intermediair 18 (26): 25-29.

X Een beoordeling van Nederlands wetenschappelijk onderzoek die niet nadrukkelijk rekening houdt met de beperkingen van ons grond- en taalgebied, is een dorpse bezigheid.

XI Het verplichte lijstje met stellingen heeft als graadmeter voor de algemene wetenschappelijke kompetentie van de promovendus slechts betrekkelijke waarde.

XII Het bouwplan van een mier blijft vernuftiger dan dat van een ruimteveer.

Leo Vroman, 1966. 126 Gedichten: 77.

XIII Ook in het kader van het deltaplan dient men zich te realiseren dat De Zeeuwse Leeuw moeilijk ontkomt aan water vervuild als dat van de huidige Wester-Schelde.

Marinus J. Sommeijer
Social mechanisms in stingless bees
Utrecht, 7 december 1983

"Ainsi, sachant que les Méliponides font de la cire et qu'elles l'emploient à la construction de leurs ruches, nous connaissons leur but, mais nous ignorons leurs moyens."

M. SPINOLA (1840). *Annales Sciences Naturelles* II: 116-140.

Aan mijn ouders
Aan Leni, Dirkje
en Tine

Omslag: Een werkster van *Melipona favosa* legt een ei in een broedcel. Dit ei wordt, zodra de werkbij zich van de cel verwijdert, opgegeten door de koningin.

INHOUD

ALGEMENE INLEIDING EN SAMENVATTING

A. De betekenis van sociale insekten voor sociaal-gedragsonderzoek

De sociale insekten spelen in veel delen van de wereld een belangrijke rol bij de bepaling van het natuurlijk milieu. Zo vormen b.v. mieren en termieten samen reeds 30 procent van de totale biomassa in het Amazone-gebied, en verzetten daar, evenals in andere tropische streken, meer aarde en humus dan de aardwormen voor hun rekening nemen (BRIAN, 1983; WILSON, 1982).

Dat naast het oecologisch gericht onderzoek ook andere biologische vakgebieden de laatste jaren veel aandacht hebben besteed aan de sociale insekten is echter vooral een gevolg van de bijzondere geschiktheid van deze dieren voor experimenteel, observationeel en theoretisch onderzoek van sociaal gedrag. Zij lenen zich met name voor onderzoek naar de evolutie van specifieke aspekten hiervan, zoals b.v. opofferend gedrag ("altruïsme").

Belangrijke theoretische modellen hieromtrent, zoals b.v. die over verwantschapsselektie ("kin selection"), zijn in belangrijke mate gebaseerd op onderzoek aan sociale insekten. Ondanks de geruchtmakende diskussie over sociobiologische modellen gepresenteerd in de zeventiger jaren, is het onmiskenbaar dat er belangrijke overeenkomsten zijn in de sociale organisatie van deze insekten en die van andere in groepen levende dieren. Zo kan de studie van deze insekten, als we de grote verschillen met andere sociale soorten in acht nemen, nieuwe informatie leveren over kondities en drijvende krachten voor de evolutie van sociaal gedrag in het algemeen. De bijzondere bijdrage van de insekten is hierbij de betrekkelijke inzichtelijkheid in details van hun sociale struktuur en het zeer grote aantal soorten dat voor vergelijkend gedragsonderzoek beschikbaar is.

De hoog sociale ("eusociale") insekten (mieren, termieten en enkele bijen- en wespensoorten) bezitten in hun kolonie-organisatie drie belangrijke kenmerken: a. samenwerking van individuen bij de broedzorg, b. taakverdeling bij de voortplanting (specifieke geslachtsdieren produceren eieren terwijl andere specialisten de juvenielen verzorgen en voeden), c. verschillende generaties overlappen elkaar.

Tussen soorten met dit eusociale nivo en soorten met een strikt solitaire levenswijze, worden verschillende vormen van minder komplekse kolonie-organisatie gevonden. Deze verschillende nivo's van socialiteit, die we bij recente soorten aantreffen, kunnen ook, indien gerangschikt in orde van toenemende kompleksiteit, gezien worden als evolutionaire stappen in de ontwikkeling van eusocialiteit (EVANS & WEST-EBERHARD, 1970; MICHENER, 1969, 1974; WILSON, 1971, 1975). Er zijn twee routes voorgesteld waarlangs dit evolutionair proces zou hebben kunnen verlopen. De z.g.n. "subsociale route" is een ontwikkelingsreeks uitgaande van de situatie waarbij de nakomelingen van een vrouwtje samen met de moeder het nest blijven bewonen. Hieruit kan zich een samenwerkingsverband tussen moeder en dochters, dieren van verschillende generaties, ontwikkelen. De z.g.n. "parasociale route" gaat uit van een groepsverband dat gevormd wordt door uitsluitend dieren van dezelfde generatie, en niet noodzakelijkerwijs direkte verwanten, die door specifieke omstandigheden bij elkaar gebracht worden.

B. Het voorkomen van eusociaal gedrag bij de bijen

Hoewel de meeste bijensoorten (Apoidea) solitair leven, vinden we voorbeelden van de ontwikkeling tot sociaal gedrag in 5 van de 9 families van deze superfamilie. De hoogste nivo's van sociale organisatie treffen we vooral aan binnen de Apidae (MICHENER, 1974). Binnen deze familie vormt de bekende honingbij, *Apis mellifera*, met slechts vier andere *Apis* soorten de Apinae. De hommels en de prachtbijen vormen samen de subfamilie van de Bombinae, en tenslotte is er de subfamilie van de angelloze bijen, de Meliponinae. Hommelkolonies zijn op het toppunt van hun jaarlijkse ontwikkelingscyclus primitief- eusociaal. Echter, doordat iedere kolonie gesticht wordt door één enkele solitair overwinterde koningin, doorloopt de hommelkolonie in haar éénjarig bestaan een ontwikkelingsreeks van solitair via subsociaal naar primitief-eusociaal verband.

Het feit dat hoog eusociale kolonies alleen voorkomen bij honingbijen en angelloze bijen, heeft ertoe bijgedragen dat men binnen de Apidae deze twee subfamilies filogenetisch nauw verwant achtte. Recent onderzoek heeft echter duidelijk gemaakt dat de Meliponinae al eerder zijn afgescheiden van een nog gemeenschappelijke Bombinae/Apinae stam (WINSTON & MICHENER, 1977).

C. De maatschappelijke en biologische betekenis van de angelloze bijen

De angelloze bijen zijn bijzonder talrijk, in soorten en aantallen, in uitgestrekte gebieden van tropisch Amerika en zuid-oost Azië. Ze komen verder uitsluitend in andere tropische gebieden voor. Er zijn twee theorieën omtrent het gebied van waaruit ze zich verspreid hebben. De meest acceptabele gaat uit van een centrum van oorsprong en dispersie in Afrika (WILLE, 1983).

Deze bijen worden onder de belangrijkste bestuivers van de tropen gerekend (NOGUEIRA-NETO, 1970; WILLE & MICHENER, 1973). Hun bestuivingsrol is met name in Centraal- en Zuid-Amerika onderkend (MICHENER, 1973). Hoewel de honingbij, *A. mellifera,* al door de vroege Europese veroveraars is ingevoerd in de Amerika's, heeft deze zich in tropisch Amerika nooit goed in het wild kunnen ontwikkelen. Pas na de recente invoer van een Afrikaans honingbijras, en de "ongekontroleerde" hybridisering van aanwezige gedomesticeerde honingbijen hiermee, heeft de honingbij zich sterk verspreid (MICHENER, 1975).

"Meliponiculture", bijenteelt met angelloze bijen voor de produktie van honing en was, is nog steeds algemeen in tropisch Amerika, en was van grote betekenis in de pre-Columbiaanse kulturen van Maya's en Azteken (WAGNER, 1960). Modernisering van dit inheems bedrijf blijft echter achterwege, vanwege de gemiddeld lagere honingopbrengst in vergelijking met bijenteelt met *A. mellifera* (NOGUEIRO-NETO, 1951, 1970).

Wetenschappelijk zijn de angelloze bijen vooral ook van betekenis door hun komplekse vorm van sociaal gedrag, die in veel opzichten de hoog sociale struktuur van *A. mellifera* evenaart en overtreft. Het is voor de studie van sociaal gedrag met name interessant dat deze groep zo soortenrijk en zo variabel is. Er zijn enkele honderden soorten beschreven in tegenstelling tot de honingbij waarvan slechts 5 soorten zijn bekend. De soorten variëren sterk zowel in lichaamsgrootte als in aantallen bijen per kolonie, maar ook in de architektuur van het nest en keuze voor de nestplaats. Hoewel de meeste soorten hun larven voeren met het typische bijen-dieet, stuifmeel en honing, is er ook een soort beschreven die zich obligaat voedt met kadavers (ROUBIK, 1982).

Ondanks de variabiliteit binnen de groep, blijkt het sociale gedrag specifieke overeenkomsten te vertonen. Dit betreft met name het gedrag rond het eileggen van de koningin. Het eileggedrag ("ovipositie") en het voeren van de larven is zeer verschillend van dat gedrag bij de honingbij. Het is door meerdere auteurs, met name door Sakagami en Zucchi, onderzocht bij verschillende soorten uit Brazilië. SAKAGAMI heeft recentelijk (1982) de verkregen resultaten samengevat. Enkele belangrijke verschillen met de honingbij, m.b.t. het voortplantingsgedrag in het nest, betreffen de drie volgende kenmerken:

a. Larvale voeding door cel-bevoorrading ineens

De larven worden niet zoals bij de honingbij doorlopend en in toenemende mate naarmate ze groter worden (progressief) gevoerd, maar door een eenmalige massa-bevoorrading van de broedcellen. Dit vullen van de broedcel gaat vooraf aan het eileggen. De cel wordt na de ovipositie afgesloten, waardoor er geen intensief kontakt is tussen opgroeiende larven en voedsterbijen. De cellen worden eenmalig gebruikt en er moeten dus voortdurend nieuwe cellen worden gebouwd.

b. Ritmiek van het eileggedrag

Het verloop in de tijd van het cel-bevoorradingsgedrag van de werksters en het eileggen van de koningin daarna, is voor een aantal soorten gedetailleerd beschreven. Dit verloop heeft bij de meeste soorten een typisch cyclisch karakter. Broedcellen worden gebouwd tijdens de z.g.n. "extra-oviposition" periode. Het bevoorraden, beleggen en afsluiten van de cel wordt in een aaneengesloten gedragsproces uitgevoerd in de z.g.n. "oviposition" periode. Deze laatste keten van gedragingen wordt het "provisioning en oviposition process", afgekort het "POP" genoemd. Het temporele verloop van dit gedrag is opmerkelijk. Alle POP-gedragingen van één cyclus worden in relatief korte tijd uitgevoerd. Dit is mogelijk door de synchrone verhoging van de aktiviteit van een beperkt aantal werksters en de koningin. De "samenwerking" van deze dieren bestaat uit specifieke interakties tussen koningin en werksters en werksters onderling. Door dit verhoogde aktiviteitspatroon lijken de dieren tijdens het "POP" opgewonden.

Het specifieke verloop van POP gedragingen resulteert in het typisch cyclische eilegpatroon: grote aktiviteit tijdens korte POP perioden, wisselt af met rustiger gedrag in de veel langere "extra-oviposition" periodes. Deze ritmiek is zéér karakteristiek voor alle angelloze bijensoorten. In soorten met een grote dagelijkse eiproduktie wordt deze ritmiek behouden door het bouwen en bevoorraden van broedcellen in groepen tegelijk. Een aantal cellen wordt dan tegelijkertijd gebouwd. Pas als deze klaar zijn, volgt de snelle, vaak ook gelijktijdige, bevoorrading van deze cellen door de werksters, en het beleggen ervan.

c. Leggende werksters

Het leggen van eieren door werksters in aanwezigheid van de koningin is een algemeen verschijnsel bij een groot aantal angelloze bijen. Dit fenomeen is echter niet in alle soorten even duidelijk (TERADA, 1974). Werkster-ovipositie kan op twee verschillende momenten tijdens het POP voorkomen:

1. Volgend op de bevoorrading met larvaal voedsel. Het door de werkster gelegde ei wordt opgegeten door de koningin die hierna haar eigen ei in de cel legt. In enkele soorten heeft het werksterei dat op dit moment gelegd wordt de vorm van een groot, kernloos voedingsei, een z.g.n. "trophisch ei".
2. Onmiddellijk nadat de koningin heeft gelegd. De werkster die de cel afsluit, legt een ei naast dat van de koningin, alvorens de cel dicht te vouwen. Dit werksterei is levensvatbaar en ontwikkelt zich tot een mannelijke larve. De ontwikkeling van deze haploïde larve is sneller dan, en gaat ten koste van die van de diploïde vrouwelijke larve. Het resultaat is dat er een mannetje uit deze cel komt.

De hierbij genoemde aspekten van de biologie van angelloze bijen geven al aan hoe basaal verschillend hun sociale organisatie is in vergelijking met die van de honingbij. De betekenis van deze diergroep voor het onderzoek naar de evolutie van sociaal gedrag mag hiermee tevens duidelijk zijn. Omdat deze betekenis voor de betreffende theorievorming al geruime tijd is onderkend (KERR, 1969; MICHENER, 1974) is het opmerkelijk dat er nog steeds zo weinig over het sociale gedrag van deze dieren bekend is (SEELEY, 1982). De belangrijkste vragen m.b.t. de socialiteit van deze dieren betreffen uiteraard de regulatie van het voortplantingsgedrag. Hoe en door wie worden er nakomelingen geproduceerd? Hoe wordt de produktie van koninginnnen en mannetjes geregeld en hoe worden nieuwe kolonies gesticht?

D. Dit onderzoek

In dit proefschrift wordt ingegaan op enkele aspekten van de sociale organisatie van de angelloze bijen. De meeste aandacht is gegeven aan het gedrag van individuele werksters en aan dat van de koningin. Het onderzoek is vooral gericht geweest op *Melipona favosa*, een soort die voorkomt in het noordeliik deel van Zuid-Amerika. en leeft in kleine kolonies van ongeveer 300 bijen. Belangrijke methoden bij het onderzoek waren het merken van alle leden van de kolonie met kleine rugnummers en het gebruik van een video-opstelling. Vooral toen het mogelijk werd om alle bijen van een kolonie te merken op de dag van hun geboorte konden veel nieuwe gegevens verzameld worden.

Allereerst werd gevonden dat er een zéér sterke koppeling is van bepaalde broedverzorgende taken van een individuele werkster. Van de werkbijen die betrokken zijn bij de bouw van een bepaalde broedcel zijn er enkele die een groot deel van die taak op zich nemen. Met name deze aktieve bouw-bijen bleken zodra de bouw klaar was ook betrokken te zijn bij het vullen van deze cel met vloeibaar larvaal voedsel. De taakverdeling met betrekking tot het bouwen van cellen en het voeren van de larven is dus anders geregeld dan bij de honingbij. Honingbijwerksters vervullen al naar hun leeftijd een reeks van verschillende broedverzorgingstaken die afhankelijk zijn van de tijdelijke aktivering van specifieke kliersystemen. Ook bij de angelloze bijen blijkt de taakverdeling onder de werksters toch afhankelijk van de leeftijd. Jonge werkers vertonen nestaktiviteiten en de oudere werksters fungeren als haalbijen.

Het leggen van eieren door "moergoede" werksters komt zeer algemeen voor. In ongeveer de helft van de cellen volgt er op het vullen van de cel met larvaal voedsel eerst eileg door een werkster. De ontwikkeling van de werksterovaria is leeftijdgebonden. Alleen in de bijen van de leeftijdsgroep die ook eieren leggen, worden hoogontwikkelde ovaria gevonden. De bijen die cellen bouwen en bevoorraden met larvaal voedsel behoren tot de categorie die ontwikkelde ovaria bezit. De eieren worden vooral gelegd aan het eind van de periode dat een werkster betrokken is bij de bouw en de bevoorrading.

Het onderzoek naar de doorgifte en uitwisseling van vloeibaar voedsel ("trophallaxis") tussen werksters, maakte verder duidelijk hoe deze bijen ook in dit gedrag verschillen van de honingbij. Alle voedseldoorgiftes tussen twee dieren begonnen met het bedelen van één van de partners. Het spontaan aanbieden van vloeibaar voedsel wordt alleen vertoond door terugkerende nectar-haalsters. Bedelgedragingen kunnen worden beloond, maar blijven ook vaak onbeloond. De bijen die op een bepaald moment de belangrijkste bevoorraders zijn van broedcellen, blijken veel te bedelen en relatief vaak beloond te worden. We noemen ze "dominant" in het trophallaxis-gedrag. Zij kunnen zelfs, als ze tijdens de kortdurende bevoorrading van een bepaalde broedcel al één keer larvaal voedsel in de cel hebben gebracht, tijdens het verloop van de bevoorrading nog snel voedsel "bijtanken" bij andere werksters en zo nogmaals een keer hun honingmaag leegpersen in deze cel. De duidelijke gerichtheid van de voedselstroom naar deze celbevoorraders is in tegenspraak met de gedachte dat het netwerk van de vele voedseluitwisselingen binnen een sociale insektenkolonie onbeperkt in alle richtingen zou verlopen (het "sociale maag" principe).

De interakties tussen koningin en werksters werden bestudeerd in relatie met de verplaatsing van de koningin door het nest en het verloop van celbouw en celbevoorrading. Het gedrag van de koningin weerspiegelt sterk de ritmiek van de lange periodes met uitsluitend celbouw afgewisseld met de korte periodes met bevoorradings- en eileggedrag. Tijdens de celbouwperiode zit de koningin meestal stil op een z.g.n. rustplaats, betrekkelijk ver verwijderd van de plaats waar nieuwe cellen worden gebouwd. Soms komt ze af en toe bij de cellen in aanbouw, maar vaak komt ze pas als er een nieuwe cel klaar is om voorzien te worden van larvaal voedsel. De koningin speelt bij dat bevoorradingsgedrag een zeer belangrijke rol. Zij steekt enkele keren haar kop in de cel en betrommelt de werksters heftig met voorpoten alvorens deze werksters de cel vullen door het leegpersen van hun honingmaag.

Evenals bij de honingbij, maar in een andere vorm, vormt er zich rond de stilzittende koningin een "hofstaat". Dit is een groep werksters die op de koningin gerichte gedragingen uitvoeren. We vonden een sterke positieve correlatie tussen de mate waarin een werkster celbouw en bevoorradingsgedrag uitvoerde en de mate waarin zij betrokken was in het uitvoeren van specifieke hofstaatgedragingen rondom de koningin op haar rustplaats. De meest aktieve bouwbijen verlaten de cel in aanbouw regelmatig om even deel te nemen aan het hofstaat gedrag rond de koningin. Die zit dan nog relatief ver van de cel verwijderd. Het aantal van deze hofstaatbijen en hun interakties neemt toe naarmate de cel verder gereed komt. Uiteindelijk gaat de koningin lopen en komt ze bij de cel die klaar is. Wij konkluderen dat dit hofstaatgedrag, dat nog enkele agressieve gedragselementen bevat en dat in enkele andere angelloze bijen tot sterk geritualiseerde "bijen-dansen" geëvolueerd is, van betekenis is voor de regulatie van het gedrag van de koningin. In die zin dient het hofstaatgedrag om de koningin "te informeren" over de mate van voortgang van de celbouw.

De betekenis van de karakteristieke cyclische verloop van het voortplantingsgedrag in de tijd is nog niet eerder verklaard. Onze hypothese is dat deze ritmiek een aanpassing is die de kontrole van de koningin over de eileg door werksters bevordert. Bij *M. favosa* als bij veel andere soorten, manifesteert de koningin haar dominantie in de eerste plaats door de eieren van werksters op te eten. De celbevoorrading komt normaliter pas dan tot stand als de koningin op de afgebouwde cel is aangekomen. Aangezien werksters alleen leggen in cellen waarin reeds larvaal voedsel is gebracht, kan er dus in principe niet in afwezigheid van de koningin door werksters gelegd worden. Door deze werkstereieren op te eten voorkomt de koningin dat de werksters nakomelingen krijgen. De evolutie van het groepsgewijs bouwen en bevoorraden van aantallen cellen tegelijk, zoals dat voorkomt bij soorten met grote kolonies, wordt in dit verband besproken.

Het meest recente resultaat dat in deze dissertatie is vermeld, betreft het voorkomen van verschillende werkstereieren in *Melipona* kolonies. Het was bekend dat in "moerloos" geraakte kolonies de werksters na een periode van meerdere dagen of weken er toch in kunnen slagen het cel bevoorradingsproces te laten verlopen. Deze cellen worden dan uitsluitend door werksters belegd. Deze legsters vertonen veel onderlinge agressie: ze duwen elkaar van de cel en eten elkaars eieren op. De laatst leggende werkster sluit zelf de cel af. Werkstereieren die onder deze omstandigheden worden gelegd, ontwikkelen zich tot mannetjes. Onder "moergoede" omstandigheden leggen *Melipona* werksters steeds voorafgaand aan de ovipositie van de koningin; deze werkstereieren worden door de koningin opgegeten. Door werkstereieren m.b.v. scanning electronen-mikroskopie te onderzoeken, vonden we dat "moergoede" en "moerloze" werkstereieren morfologisch verschillend waren. Werkstereieren uit een "moergoede" kolonie hadden een gladdere en minder stevige oppervlakte. Deze werkstereieren stonden minder stabiel op de vloeistofspiegel van het larvaal voedsel in de cel, waardoor ze minder levensvatbaar waren. Deze verschillen in kwaliteit van de werkstereieren zijn nog niet eerder beschreven voor sociale bijen. Echter, bij de mierensoort *Myrmica rubra* heeft de aanwezigheid van de koningin een soortgelijke invloed op de kwaliteit van het werksterei. De koningin "dwingt" werksters tot een vervroegde eileg. Zulke eieren dienen bij deze mieren uitsluitend als voedsel voor de larven (BRIAN & RIGBY, 1978).

We kunnen konkluderen dat de sociale organisatie van de angelloze bijen principieel verschilt met die van de honingbij. Binnen het basale patroon van hun sociale struktuur blijken de meer dan 300 soorten angelloze bijen een grote diversiteit te vertonen in de sociale regulatie van hun voortplantingsgedrag in het nest. Vooral door die onderlinge variatie vormen deze bijen een interessant objekt voor vergelijkend onderzoek van sociaal gedrag.

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DISTRIBUTION OF LABOUR AMONG WORKERS OF *MELIPONA FAVOSA* F : CONSTRUCTION AND PROVISIONING OF BROOD CELLS

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SUMMARY

The behaviour of individual workers participating in the construction and provisioning of brood cells was studied in colonies of *Melipona favosa favosa* (F.). A strong linkage at the individual level was found between the building of a brood cell and the subsequent mass-provisioning of this cell with larval food : the most active builders of a brood cell were also the most important provisioners of that cell. All bees that participated in the provisioning of a cell (5-8 individuals) had previously worked on construction of that cell. In addition to the building-provisioning bees, other workers were exclusively building workers. The building-provisioning workers, however, are characterized by their distinctly higher individual building activity and by the fact that they increase their building activity considerably in the final stages of construction.

Some general aspects of the provisioning and oviposition process and the behaviour of queen and workers are mentioned.

RESUME

Distribution du travail parmi les ouvrières de Melipona favosa F : construction et approvisionnement des cellules à couvain

Le comportement individuel des ouvrières participant à la construction et l'approvisionnement des cellules à couvain ont été étudiés dans des colonies de *Melipona favosa favosa* (F.).

Nous avons trouvé une forte relation entre la construction d'une cellule à couvain et l'approvisionnement massif de cette cellule en nourriture pour les larves : les constructrices les plus actives d'une cellule à couvain étaient aussi les approvisionneuses les plus importantes de cette cellule. Toutes les abeilles participant à l'approvisionnement d'une cellule (5-8 individus) avaient participé auparavant à la construction de cette cellule. A côté des abeilles constructrices-approvisionneuses, d'autres ouvrières sont exclusivement constructrices. Les ouvrières constructrices-approvisionneuses se caractérisent par une activité de construction plus importante et par une augmentation importante de leur activité de construction au cours des derniers stades de construction.

Nous rapportons quelques aspects généraux de l'approvisionnement, de l'oviposition et du comportement de la reine et des ouvrières.

INTRODUCTION

Two tribes of the Apinæ, the Meliponini, the stingless bees, and the Apini, the true honey bees, possess the same high level of social organization. However, with respect to the reproductive behaviour within the nest, there is a marked contrast between the mass provisioning of the stingless bees and the progressive feeding by the Apini.

The group of stingless bees has a pantropical distribution and comprises a few hundred species. The importance of this group for the study of the evolution of social behaviour in insects is recognized. The differences between the nest architecture of the species — regarded as a form of " cristallized behaviour " — (Evans and West Eberhard, 1973) have been investigated intensively and have been used as a base for classification and evolutionary studies (Wille and Michener, 1973 ; Michener, 1974). The nest of most species contains an arrangement of brood cells in horizontal combs. In other species the brood cells are positioned in clusters ; few species are known to arrange their brood cells in intermediate arrangements. The brood chamber of comb-building species is surrounded by a structure of waxy sheaths. This involucrum separates the brood chamber from the rest of the nest where the ovoid food storage pots are located. In a series of publications by Sakagami and associates from 1963 onwards, the brood cell provisioning and oviposition process (POP, Sakagami, Montenegro and Kerr, 1965) has been described in detail for about 20 species of stingless bees *(Meliponini)*. Many important aspects of stingless bee biology are revealed in this comprehensive study, e.g. laying worker activity, feeding of the queen and the unique temporal articulation of the oviposition process. Sakagami and Zucchi (1974) have reviewed the cell-building and oviposition for the species studied so far. They classified cell construction into 3 types : 1. successive : various cell stages are found at a given time ; 2. synchronous : cells are started synchronously and cells found at a given time are all at the same stage ; 3. semi-synchronous : cells tend to be started successively but differences become gradually smaller. The species could also be classified on the basis of the typical succession pattern of their ovipositions, e.g. exclusively batched, facultatively batched or predominantly singular.

All species of the genus *Melipona* are comb builders. The general pattern for comb building and oviposition in *Melipona* is as follows : the brood cells, which are used only once, are constructed at the margins of the combs (advancing edges). The first cells of a new comb are generally built centrally above the previous comb or centrally at the bottom of the nest cavity. Brood cells are ready for oviposition when they are provided with a smooth collar that protrudes above the neighbouring comb surface. At this stage a group of workers in the vicinity of the cell may gradually become rather excited : they agglomerate around the structurally ready cell, making frenetic body movements and alternately inserting their fore-bodies briefly into the cell. These activities increase gradually until the queen arrives and they may continue for some time while frequent interactions occur between queen and workers. The inserting workers subsequently discharge larval food into the cell by contracting their abdomen. After these body insertions with distinct abdominal contractions a number of insertions without contraction may occur. Once the cell has been filled to about two thirds of its capacity the queen oviposits centrally on the surface of the larval food. Prior to the oviposition of the queen a worker oviposition may occur ; generally the worker's egg is immediately devoured by the queen. In addition to this oophagy the queen may feed on some of the larval food. The latter type of feeding is also common in the absence of worker oviposition.

We have attempted to gain further insight into the social organization of stingless bees especially with respect to the roles that the queen and individual workers play in reproductive activities within the nest. In this paper we concentrate on the behaviour of workers involved in the building and provisioning of brood cells. The study was made on *Melipona favosa favosa* (F), a species whose behaviour is so far unknown. The advantage of this species is that the number of workers in the nest is relatively low, a factor which facilitates study of the roles of individual bees.

MATERIAL AND METHODS

Most of our observations were carried out on four colonies from Surinam kept at the Laboratory of Comparative Physiology of the University of Utrecht. Additional observations were made in Trinidad, West Indies, on colonies kept in observation boxes but with free exits to the outside. After colonies had been collected in the field they were transferred to transport boxes provided with exit holes. To facilitate adaptation they were kept in these boxes for several weeks before they were flown to Utrecht. Here each was transferred to an observation hive, a modification of the type described by SAKAGAMI (1966), the major difference being that the inner box is divided into two compartments. One part contains the nest and a larger area permits the bees to wander around and to collect sugar syrup. A pollen mixture (prepared after CAMARGO, 1976) was fed to the bees daily in the nest compartment. All workers were marked (on the thorax) on the same day with small stickers with letter codes for individual recognition. Special care had to be taken that this tag did not noticeably influence the behaviour of the bee. The bees that hatched from brood cells after the time of marking were left unmarked in the colony.

Observations were carried out until the first unmarked bees were seen to be involved in building and provisioning activities. The behaviour was observed directly at the observation hive or was studied with the help of video equipment; in all cases only red or far red light was used. We were able to follow the progress of the brood cell construction, noting the height of the developing cell in terms of a four-part construction process up to the level of the comb: a "4/4 stage" thus indicates a developing cell attaining the level of the comb surface. In addition, we use the term "c-stage" to denote the cell when the protruding collar is completed. We studied the building behaviour in two ways. Firstly there were short momentary samples in which it was established which animals were involved in building activity. These recordings yielded information about the frequency with which individuals participated in the construction of a particular cell. The relative contribution of each individual bee to this process could thus be determined. In a later series the behaviour was continuously observed during observation periods of fixed length. During these the building time of each participating worker was measured. In this series, periods of 10 min. observation alternated with periods of 15 min. non-observation per cell. However, if three or four cells were under construction at the same time, this schedule was changed to a sequence of 5 min. observation and 15 min. non-observation per cell. The observation schedule for a certain colony was started immediately after its members had been marked and the observations were continued uninterruptedly for periods of 4-8 days. During these observation series a number of cell provisionings (POP's) took place; these were all recorded on video tape and analysed later with respect to the kind and the intensity of involvement of the participating bees. The involvement of individual bees in cell provisioning was compared with the involvement in building by the same individuals. Detailed observations were carried out on the comparatively strong colonies F9 (two series, in January and March, 1978 resp.) and F78-2 (April, 1978). The workers of a third colony (F8) were not marked individually; this colony was used only for general observations on oviposition rate, etc. A fourth colony (F5), the first that was used chronologically in october and november 1977, had experienced an interruption in brood production and at that time was declining. As a result no young bees emerged during the observation periods. The data from this colony were not used since irregularaties were expected.

RESULTS

Before we give the results of our observations concerning cell building and provisioning, we shall mention some general aspects of the provisioning and oviposition process and the behaviour of queen and workers.

1. Colony size

The moment the colonies arrived in Utrecht the conditions within the colonies gave us indications about the colony size and the natural conditions, matters that are important for the interpretation of the results obtained with colonies in captivity. These data are presented in *table I.* The table shows that those colonies that were used for observations in 1977 and 1978 contained on the average equal numbers of adult bees and immature stages which ranged from 100 to 200 per colony. The colonies which arrived in 1979 were slightly larger in adult population but contained considerably more brood cells.

Table I. — Size of the colonies upon arrival in Utrecht. The colonies 5, 9 and 78-2 were used for the observations on individual bees.

Tableau I. — Dimension des colonies au moment de leur arrivée à Utrecht. Les abeilles ont été observées individuellement dans les colonies n° 5, 9 et 78-2.

Colony	Arrival date	Origin	Estimated number of workers	Estimated number of brood cells
5	11-04-1977	Surinam	85	75
9	07-12-1977	Surinam	150	150
8	07-12-1977	Surinam	100	170
78-2	11-01-1978	Surinam	180	75
79-2	13-08-1979	Trinidad	250	370
78-3	13-08-1979	Trinidad	230	530
79-4	15-10-1979	Surinam	150	680

2. Oviposition rate and duration of cell construction

The oviposition rates during the observation periods in 1977 and 1978 were lower than the rates observed in Trinidad and in Utrecht in 1979. The results are presented in *table II.* The data reveal an average of 2.9 daily ovipositions for the experimental colonies and 4.2 for the unmarked colony, if we exclude F5, a declining colony (Material and Methods). In 1979 we obtained averages for colonies in Utrecht and Trinidad ranging from 7.3 to 15.4 daily ovipositions. These results illustrate the low productivity of this species in comparison with the honey bee, *Apis mellifera,* where the queen

Table II. — Oviposition rate. The results for the colonies TR. 1-4 are data obtained for the free-flight colonies in Trinidad ; the other data relate to colonies kept in captivity.

Tableau II. — Mesure du taux d'oviposition. Les colonies TR. 1-4, étudiées à Trinidad, avaient liberté de vol. Les autres colonies ont été étudiées en captivité.

Colony	Average daily oviposition	Observation period (days)
5	1.2	39
8	4.3	42
8	4.0	7
9	2.7	34
9	3.2	9 *
9	2.4	5 *
78-2	3.2	4 *
79-2	9.8	46
79-3	8.7	47
79-4	7.3	49
TR.1	15.3	125
TR.2	15.4	51
TR.3	14.3	92
TR.4	11.3	37

* Periods of observation on behaviour of individually marked bees in 1977 and 1978.

may lay 1000 to 2000 eggs daily. It should be stressed that the data for the marked colonies resulted from observations taken during the first few days after all bees from the colony had been marked, a process which caused significant disturbande. They may represent an under-estimation of the natural oviposition rate.

Several brood cells may be under construction at the same time. These cells are not built synchronously ; different building stages may occur at the same time. Cells are generally provisioned one by one at considerable time intervals. This in contrast to some other species where a batch of cells is provisioned and completed simultaneously. However, in Trinidad on several occasions two ovipositions in rapid succession were observed under the prevailing favorable conditions. The simultaneous occurrence of more than 2 cells in the collar stage was never observed. Representative examples of the progress of the construction of cells are given in *figure 1*. As can be seen from this illustration the starting of new cells is rather irregular. New cells may be started while other cells are in various stages of development. *M. favosa* is thus a typical successive cell-building species. The construction time was variable, due to delays which occur in different development stages. The average time needed for the construction of a single cell, as determined for the two last observation periods in Utrecht, was 13 hours and 10 hours respectively.

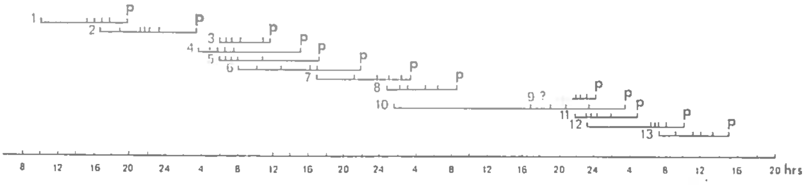

Fig. 1. — The progress of the construction of 13 successive brood cells. Horizontal bars represent the duration of the construction of a cell until provisioning starts. Short vertical bars demarcate the (estimated) respective four parts of the growth in height of the cell. A " p " at the end of a horizontal bar indicates the process of provisioning and oviposition for that cell.

Fig. 1. — L'évolution de la construction successive de 13 cellules à couvain. Les barres horizontales représentent la durée de la construction de la cellule jusqu'à l'approvisionnement. Deux barres verticales délimitent les quatre moments de construction en hauteur (estimés) de la cellule. Un « p » à la fin d'une barre horizontale indique le début de l'approvisionnement de la cellule et de l'oviposition dans cette cellule.

In Trinidad the delays were less prevalent ; there the average building time was estimated to be 4-5 hours.

3. General remarks about provisioning, oviposition and cell operculation

a) *Behavioural elements of the queen*

The queen possesses a characteristic behavioural repertoire. When walking or when at rest she may initiate a type of interaction with a worker which is commonly referred to in literature as " food-begging " : when being approached by, or when approaching a worker frontally, she grabs both sides of the head of the worker with her front legs and antennae. The worker responds in most cases with a crouching behaviour making a bow by turning her vertex to the queen. Only in a few instances does the worker respond by offering food to the queen. She also engages in characteristic wing beats, consisting of simple strokes of irregular, low frequency ; and she frequently draws one of her hind legs along the dorsal and lateral sides of her abdomen. This typical stroking with the hind legs was likewise observed in *M. favosa* colonies in Trinidad. In addition, we observed this behaviour in colonies of *M. beecheii, M. scutellaris trinitatis, Paratrigona* sp. and *Trigona (Tetragona) nigra paupera.* ZUCCHI (pers. comm.) and SAKAGAMI (pers. comm.) confirmed that this element also occurred in other stingless bees.

During periods between ovipositions the queen was away from the advancing edge most of the time. She was usually on the floor of the hive under the brood nest or storage pots. She generally remained there in one place or in several definite places. From there she undertook short round-trips in the nest, during which she regularly visited the advancing edge. Such excursions became more frequent when the building of the most advanced brood cell reached its final stage. In periods of relatively frequent ovipositions she made fewer visits per new cell. Then her visits coincided more or less with the completion of each cell. When the queen approaches the cell she may perform a behaviour commonly referred to as " inspection behaviour " : she inserts her head and part of her thorax into the cell. These " inspections " may be carried out during different stages of the cell contruction. However, they are most common in the final construction stages immediately before and during provisioning. After the queen arrives at a brood cell with a smooth collar, she performs fast drumming movements (ruffle beatings) with her front legs and antennae on the dorsal parts of the head, thorax and abdomen of workers which are around the cell and are performing body insertions. As will be discussed in a later paragraph, such body insertions may pass into food-dischanges by the workers and occasionally a worker egg may be laid at the end of this sequence. Such workers' eggs that are laid at the end of provisioning are eaten by the queen. The queen always takes up some larval food immediately before oviposition. After laying, the queen returns to the floor of the hive.

We have made some quantitative measurements of the behaviour of a queen. We observed her continuously for 4 periods of 30 minutes each. These periods were chosen in each of the different stages of cell construction.

Frequency and duration of certain behavioural elements are shown in *table III.* The table suggests that the time spent by the queen on the comb increases as the construction of the brood cell progresses. This is certainly true for the number of ruffle beatings on workers near the cell and the number of food beggings. The frequent begging for food by the queen is only rarely rewarded. This was revealed by another series of observations on the behaviour of the queen in which her visits to the comb were recorded from the time that the most advanced cell was provided with a collar until provisioning started. In that rather delayed period of several hours only 6 percent of her frequent food beggings were rewarded.

Table III. — Frequency and duration of certain elements of the queen's behaviour during 5 observations of 30 minutes each, at different stages of cell development.

Tableau III. — Fréquence et durée de certains éléments du comportement de la reine pendant 5 observations de 30 minutes, chacune à des stades différents du développement des cellules.

	Observation 1	Observation 2	Observation 3	Observation 4
Height of cell relative to comb	no cell under constr.	2/4	4/4-C	C-stage
Number of visits to the comb	0	4	3	4
Duration of stay on the comb (sec.)	0	648	876	1 450
Number of food beggings	25	34	65	72
Number of ruffle beatings on workers	0	1	6	54
Number of cell-inspections	0	5	1	7

b) *Provisioning by the workers*

When the construction of a cell is completed there are always a few workers around the cell who are already performing brief body insertions when the queen arrives. During the body insertions of a worker the queen may drum the worker on her dorsal body parts. A distinct contraction of the abdomen indicates the first discharge of larval food by one of these workers. In general, successive discharges follow immediately, resulting within a few minutes in the filling of the cell up to about two thirds of its volume. In between the " actual " discharges (body insertions with abdominal contractions) there are also some body insertions without abdominal contractions. These types of insertions were more frequent at the end of the provisioning. The number of discharges was determined for each POP (provisioning and oviposition process) recorded and the averages for the various colonies are given in *table IV.* The average numbers of discharges observed in Utrecht and Trinidad appeared to be rather similar with one notable exception. Colony F9 in Utrecht demonstrated a distinctly higher number of discharges in the two observation series ($\bar{x}$ = 16.6 and 17.5), which were 2 months apart ; otherwise the means were from 10.5 to 12.4.

c) *Worker oviposition and cell operculation*

Altogether 61 POP's were recorded on videotape. These were used to determine the frequency of worker ovipositions. It appeared that no worker ovipositions occurred in the declining colony F5. In 21 of the 43 POP's in the other colonies only one worker oviposition occurred and 2 worker ovipositions in the same cell were exceedingly rare, only in one POP was this recorded. From direct observations on captive colonies and from observations in Trinidad we gained the same impression. Among 260 observed POP's in Trinidad, 58 % contained one or more worker-ovipositions, 13 % contained 2 worker-ovipositions and 3 worker eggs were laid in 1.5 % of the observed POP's. Although the worker oviposition always occurs near the end of the discharging sequence, it does not necessarily terminate this sequence. The occurrence of one or more final discharges was commonly observed following oöphagy by the queen. Worker ovipositions after oviposition by the queen were never observed in these cases.

The sealing of the cell after the queen has laid is similar to that reported for other *Melipona* spp. The operculation is started by a single worker soon (5-10 seconds) after the withdrawal of the queen. The worker closes the cell, folding the collar inwards while rotating her body over the aperture with her abdominal tip inserted. When the cell is nearly closed, she withdraws her abdomen from the aperture and continues the folding from the side. At that stage she may be assisted by one to several other workers.

4. Observations on building and provisioning by individual bees

For three observation series of 10, 12 and 13 successively built cells respectively, the average numbers of building individuals per cell were 13.0, 15.2 and 20.2 workers. The first two series of observations were both made on colony F9. Although these two series were based on different methods of recording (see : Materials and Methods), the results do not differ much. This indicates that although no continuous observations were made, and probably not all participating bees were registered, this factor is of minor importance. The total number of food discharges for a given cell was performed by a limited group of about 6 workers several of which carried out more than one discharge *(table IV)*. The data from Trinidad indicate that these subsequent discharges by single bees are also common under natural conditions. The average number of discharges per cell was between 10.5 and 17.5 for the colonies in captivity. The average obtained in Trinidad is between these limits, but the number of participating bees is somewhat higher. Often the individuals who participated in discharging did so for a number of successive cells. It was also remarkable that the first discharging workers were the same for several successive cells.

Table IV. — Number of food discharges per cell and number of discharging individuals (body insertions without abdominal contraction omitted).
n : number of cells ; x : average number of discharges ; Sx : estimated standard deviation ; y : average number of individuals discharging ; Sy : standard deviation.

Tableau IV. — Nombre de dépôts de nourriture par cellule et nombre d'individus qui déposent de la nourriture.
n : nombre de cellules ; x : nombre moyen de dépôts ; Sx : déviation standard de x ; y : nombre moyen d'individus qui déposent de la nourriture ; Sy : déviation standard de y.

Colony	n	x	Sx	y	Sy
5	18	12.4	4.4	5.2	1.0
9	18	17.5	3.2	5.5	1.4
9,2nd series	12	16.6	5.8	6.3	1.7
78-2	13	10.5	2.0	6.5	1.1
TR.5	152	11.4	3.2	8.5	1.3

5. The occurrence of combined constructing and provisioning activity in workers

If we look at the constributions of the individual bees to a particular cell we find that the discharging bees were in general also constructors of the same cell (building-provisioning, BP workers). We see that only 15 % of a cell's provisioning bees have not built on that cell. However, the BP workers are not the only ones engaged in the building of cells ; they are assisted by a number of exclusively building (B) workers (*table V*).

Table V. — General labour differentiation in building and provisioning. The percentages of exclusively building (B) workers, exclusively provisioning (P) and building-provisioning (BP) workers for the scores on cells of the respective observation series are indicated.

Tableau V. — Différentiation du travail dans la construction et l'approvisionnement. Le tableau donne les pourcentages d'ouvrières exclusivement constructrices (B), exclusivement approvisionneuses (P) et à la fois constructrices et approvisionneuses (BP) pour les différentes séries d'observations.

	Colony 9 (first series)	Colony 9 (second series)	Colony 78-2
Number of cells	9	12	13
Observation period (days)	5	5	4
Number of scores	114	203	277
% of B-workers	59.6	62.6	70.8
% of P workers	5.3	7.9	2.5
% of BP workers	35.1	29.6	26.5

Not only did we analyze the data for each cell separately but we also analyzed the data for overall performance of the individuals during the whole period *(table VI)*. The results again demonstrate that nearly all discharging bees also engage in building activity. However, more than 50 per cent of all participating individuals were exclusively builders during the whole period. This may indicate that certain individuals do not participate in provisioning at any time during their lives. The behaviour of workers that demonstrated a combination of both activities for the same cell (resp. 19, 19 and 22 workers) was further specified. The regular occurrence of combined activities is obvious ; the independent occurrence of building on some of the successive cells is also common but the independent occurrence of discharging is uncommon *(table VII)*.

Table VI. — Classification of the different workers according to their overall performance during the whole observation period. The percentages of the different worker categories (B, P and BP) for overall performance are indicated for the respective observation periods. The number of cells and the observation periods are as in *table V*.

Tableau VI. — Classification des différentes ouvrières par rapport à leurs activités pendant la période d'observation. Le tableau donne les pourcentages des différentes catégories d'ouvrières (B, P et BP) pendant les périodes d'observation. Le nombre de cellules et les périodes d'observation sont les mêmes que dans le *tableau V*.

	Colony 9 (1st series)	Colony 9 (2nd series)	Colony 78-2
Total number of individuals	54	69	60
% of B workers for whole period	64.0	65.2	56.6
% of P workers for whole period	1.9	0.0	0.0
% of BP only for different cells	0.0	7.3	6.6
% of BP for at least one single cell	35.2	27.3	36.6

Table VII. — Specification of overall behaviour of those workers which performed combined building-provisioning activity at least once on a single cell. The number of cells and the observation periods are as in *table V*.

Tableau VII. — Catégorisation du comportement global des ouvrières qui ont présenté au moins une fois une activité combinée de construction et d'approvisionnement d'une seule cellule. Le nombre des cellules et les périodes d'observation sont les mêmes que dans le *tableau V*.

	Colony 9 (1st series)	Colony 9 (2nd series)	Colony 78-2
Number of individuals	19	19	22
% of B worker scores	36.3	37.2	59.1
% of P worker scores	7.0	8.2	3.5
% of BP worker scores	56.3	54.6	37.5

6. Relative building performance of the exclusively building workers and the building provisioning workers

The relative contribution of B and BP workers to the building process was investigated during two observation periods. A comparison of the individual building activity of single bees from both worker groups indicates that the individual building activity of the BP bees was distinctly higher. In addition, the BP bees are divergent in that they increase their building activity considerably in the final construction stages *(table VIII)*. A comparison of the total activity of both groups reveals that in the first construction stages the average building activity of both groups was about equal. However in the last stage the BP group had twice the total building activity of the B group *(table VIII)*. This implies that, although a considerable number of non-provisioning builders co-operate in the construction of a cell, the main part of the job is done by the small number of bees which also provision it later on.

Table VIII. — Relative building performance of B and BP workers during observation series on the colonies F9 and F78-2.

Tableau VIII. — Activités de construction d'ouvrières B et BP pendant plusieurs séries d'observations sur les colonies F9 et F78-2.

		Colony		Colony		Colony	
		9	78-2	9	78-2	9	78-2
	Stage of cell construction	Number of individuals		Aver. individual building time (seconds)		Total observed building time (seconds)	
B-workers	0 - 1/4	32	36	143	132	4570	4764
	1/4 - 2/4	23	31	134	129	3092	3997
	2/4 - 3/4	24	19	191	164	4584	3113
	3/4 - 4/4	40	31	163	170	6507	5259
	4/4 - POP	28	40	193	150	5399	5995
BP-workers	0 - 1/4	13	15	342	342	4441	4866
	1/4 - 2/4	14	12	493	343	6905	4120
	2/4 - 3/4	13	16	349	313	4537	5006
	3/4 - 4/4	15	16	756	464	11339	7431
	4/4 - POP	19	21	1187	594	22559	12478

7. Relation between the relative building and provisioning activity in individual BP bees

Ranking the builders of cells in categories according their building performance demonstrates that the categories of the most active builders contain the highest percentage of discharging bees *(fig. 2)*. For a correlation of the individual building and provisioning activity, the classification of individual provisioning performance was based on the descending order of dischar-

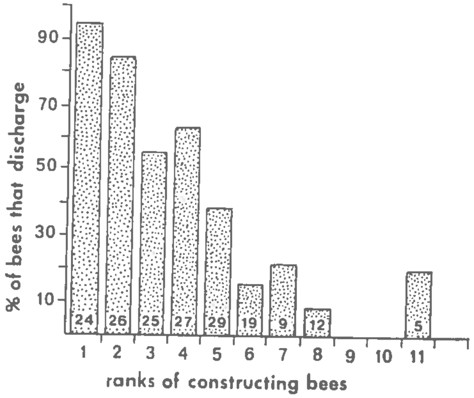

Fig. 2. — Contribution to provisioning for the different ranks of constructing bees. The constructing bees are ranked according to their building activity for the respective cells (e.g. rank 1 is formed by the most active builders for each cell, etc.). The percentages of constructing bees that contribute to the provisioning of the same cell are indicated by heights of columns (number of cases is indicated in the columns).

Fig. 2. — Contribution des abeilles constructives à l'approvisionnement en fonction de l'importance de leur activité de construction. Les abeilles constructrices ont été classées selon leur activité de construction pour les différentes cellules construites (par exemple, le rang 1 est celui des constructrices les plus actives pour chaque cellule). Les pourcentages d'abeilles qui contribuent à l'approvisionnement de la même cellule ont été indiqués pour chaque rang (le nombre de cas est indiqué dans chaque colonne).

ging individuals in a single provisioning sequence. The measured average building acticity and the average individual provisioning performance were compared for all BP bees. This yielded a high correlation between the classification for building activity and the ranking for provisioning performance *(table IX)*. Thus the most active builders are not only the animals most

Table IX. — Average relative building activity for BP workers in descending order of their discharging.

Tableau IX. — Moyenne des activités de construction des ouvrières BP, par ordre décroissant de leurs dépôts de nourriture dans les cellules.

Ranking for provisioning performance	Percentage of construction time	
	Colony F9	Colony F78-2
1	17.0	15.0
2	11.0	13.7
3	16.2	10.6
4	13.5	8.7
5	6.6	5.2
6	6.8	7.2
7	0.4	6.2
8	8.5	0.8
R (Spearman)	+ 0.83	+ 0.93

likely to discharge, but they are also the ones that discharged earliest in the sequence of discharging individuals.

DISCUSSION

Although the stingless bees are an ethologically highly diversified group (Sakagami and Zucchi, 1974 ; Sakagami, Zucchi and Portugal Araujo, 1977), the general patterns, especially the temporal aspects of POP and queen worker co-actions, appear to be generally similar in the described species of the genus *Melipona : M. compressipes manaosensis* (Sakagami and Oniki, 1963), *M. seminigra merrilæ* (Beig and Sakagami, 1964), *M. quadrifasciata anthidioides* (Sakagami, Montenegro and Kerr, 1965), and *M. rufiventris* (Camillo, 1972). *M. favosa* appears to be similar to be described species of the genus ; however, certain behavioural elements are expressed in a specific way, e.g. the distinct ruffle beatings of the queen on workers around the brood cell and the wing-beats of the queen. The peculiar stroking action of the hind-legs along the abdomen was hitherto unreported.

On observing the discrepancy in the number of worker-eggs laid in one cell by *M. compressipes manaosensis* and *M. seminigra merrilæ*, Beig and Sakagami (1964) assumed that the difference could be attributed to differences in colony conditions. We tend to think that the number of worker-laid eggs in one cell may be species-specific in *Melipona*, possibly as a result of specific differences in worker population and the dominance of the queen. Our arguments are that our results for *M. favosa* colonies in captivity as well as in Trinidad, concur with those obtained for the extensively studied *M. quadrifasciata anthidioides* (Sakagami, Montenegro and Kerr, 1965) where worker ovipositions occurred in 35 % of 129 cases. In that species, too, one worker-egg per cell was generally laid. In addition we found that the behaviour observed in captive colonies was not basically different from that in colonies flying out freely under natural conditions. This concurs with the results of Camillo Atique (1974, ref. in Sakagami *et al.*, 1977) who demonstrated the persistence of quantitative and temporal aspects of oviposition behaviour in *M. rufiventris* in captivity.

Since it is difficult to mark a considerable number of individuals without disturbing the colony, the information on the division of labour among workers of stingless bees has remained meagre. Kerr and Santos Neto (1956) introduced 8 newly emerged workers of *M. quadrifasciata* into a colony and recorded their behaviour until all died. They conclude, after comparing the observed temporal division of labour in *M. quadrifasciata* with *Apis mellifera* and *Bombus agrorum*, that *Melipona* is most like *Apis.*

An important difference concerned the wax secretion : it is more scattered in *Melipona* and is highest before the period of feeding the larvae, whereas in *Apis* larval feeding precedes wax production.

Hebling, Kerr and Kerr (1964) studied division of labour in workers of *Trigona (Scaptotrigona) xanthotricha* by taking advantage of the differences in coloration between workers of this species and those of the related *T. (S.) postica*. They introduced about 60 workers of *T. (S.) xanthotricha* as pupae, just before emergence, into a colony of *T. (S) postica*. Analyzing the principal categories of labour, they found that the construction of brood cells, wax removal from comb surface and cell operculation were performed during more or less the same period as larval feeding. Terada, Garofalo and Sakagami (1975) found for the stingless bee *Plebeia droryana* that bees which worked on the new comb and provisioned brood cells were of similar age to the comb builders. Sakagami, Montenegro and Kerr (1965) found for *M. quadrifasciata anthidioides* that workers participating in cell construction and in provisioning belonged to the same worker age group. They emphasized the distinct alternation of the builders of a cell. As a mere hypothesis for governing of cell construction process they assumed that comb workers involved in building and those participating in provisioning belong to different response groups : " building response group (cell building, comb surface, pillars, involucrum, etc.) " and " POP response group (provisioning, worker oviposition, operculation, etc.) ". They supposed that the existence of these different response groups was based on differences in " specific motivation " among the comb workers.

Our results, showing the strong linkage of the building of a brood cell with its subsequent provisioning, are not fully in agreement with this hypothesis. We conclude that the most active building and discharging bees form only one group of individuals which share a particular behavioural sequence, e.g. successive building and provisioning, because of a common specific motivational state which at low intensities facilitates building and at higher intensities leads to an intensification of building acticity and a facilitation of provisioning acticity. The fact that a limited number of bees is involved with both the construction and provisioning of particular cells further emphasizes the difference in the social organization of stingless bees and honeybees. The specific sequence of behaviour patterns in workers of stingless bees resembles the organization of the reproductive behaviour of solitary bees. It points to a particular strategy of the workers in relation to the reproductive dominance of the queen, an interesting feature in bees with such a distinct caste dimorphism.

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III THE SOCIAL BEHAVIOR OF *MELIPONA FAVOSA* F.: SOME ASPECTS OF THE ACTIVITY OF THE QUEEN IN THE NEST

M.J. Sommeijer

ABSTRACT

Stingless bees are unique eusocial insects in that they engage in mass provisioning of brood cells, an action which is behaviorally integrated with the subsequent oviposition by the queen. We investigated the behavior of the queen of *Melipona favosa* and her locomotion in the nest throughout complete periods extending from one oviposition process to another.

During most of the extra-oviposition periods the physogastric queen of *Melipona favosa* rested at fixed places, away from the young combs, and became active only shortly before the provisioning of the brood cell and the ovipositing in it. She remained on the comb for only 18% of the total observation time. She performed typical wing beats and leg-passings over her body. The temporal occurrence of this behavior was recorded. It is assumed that these behavioral elements are of importance for the dispersion of pheromones. The queen's trophallactic interactions with workers were always initiated by her begging. The temporal occurrence of this soliciting and the degree of rewarding was measured. Since only 1.8% of the solicitations was rewarded, the queen's food-begging appeared unefficient. We conclude that this food soliciting behavior has evolved into a secondary, communicative function. The queen always ingested food from a brood cell during the provisioning and oviposition process. This food consisted of larval food and/or worker eggs.

INTRODUCTION

The nests of most species of stingless bees (Hym., Meliponinae) contain several brood cells which are under construction at the same time (SAKAGAMI, 1982). In the *Melipona* species these cells occur at various stages of construction at the edges of the youngest combs. Upon termination of the construction process they are filled in an integrated behavioral process involving the discharge of larval food and oviposition ("Provisioning and Oviposition Process, POP", SAKAGAMI, MONTENEGRO and KERR, 1965). The activity of the workers constructing such a cell increases gradually as cell-building approaches completion (SOMMEIJER, BEUVENS and VERBEEK, 1982). When the activity of these workers is heightened to "excitement" behavior (SAKAGAMI, 1982) the queen arrives at the cell. After some interactions with the queen, the workers start to discharge larval food into the cell. This is usually followed by one or more worker ovipositions. Their eggs are subsequently ingested by the queen, in addition to some larval food. Next she herself lays an egg in the cell. After this she retreats from the cell and the oviposited cell is operculated by a worker very shortly afterwards. By now the "excited" activity of the workers has died down and they go on constructing other cells. This behavioral cycle is very characteristic for this group of bees and is unique among all social insects (SAKAGAMI and ZUCCHI, 1974; SAKAGAMI, 1982; SOMMEIJER, 1983). The cyclic character is maintained in meliponine species that have a higher frequency of oviposition than the *Melipona* species, such as *Scaptotrigona postica* (SAKAGAMI and ZUCCHI, 1963) because the frequent ovipositions occur in batches. Although the POP behavioral sequences can take place in queenless colonies, it appears that in queenright colonies of *Melipona*, the queen has to be near the cell in order to trigger the discharge of larval food by the workers (SAKAGAMI, MONTENEGRO and KERR, 1965; VELTHUIS, 1976; SOMMEIJER and VELTHUIS, 1977).

Specific interactions between queen and worker, occurring during POP, have been described for several species (reviewed by SAKAGAMI, 1982). Certain interactions are performed when the queen is away from the young comb, during the "extra-oviposition period". The fact that the queen is "resting" during some part of this period is also indicated for several species (SAKAGAMI and ZUCCHI, 1974). Little is known about the progress of the behavior of the queen and her locomotion through the nest. In order to understand how the queen may influence the course of the afore mentioned cyclic intranidal behavior, we need information about her activity between one POP to the following one.

In order to obtain further insight into the intranidal behavior of the queen of stingless bees, we recorded her behavior continuously during various observation series in *Melipona favosa*. In this paper we will first describe the general behavior of the queen; then some quantitative data will be presented concerning her locomotive activity, the occurrence of specific behavioral elements and her trophallactic interactions with workers. Finally, we will discuss the function of certain recorded behavioral elements and attempt to give an interpretation of some of these.

MATERIALS AND METHODS

Queen behavior was studied in various colonies of *M. favosa* which were all housed in observation hives of the type described by SAKAGAMI, 1966. Some colonies were kept under controlled conditions at our laboratory in Utrecht. Other colonies were studied in their natural environment in Trinidad (West Indies). Only the latter colonies had an exit to the outside. The behavior of the queen was registered during the extra-oviposition periods by means of direct observation. The occurrence of specific behavioral elements was studied by the use of video recordings.

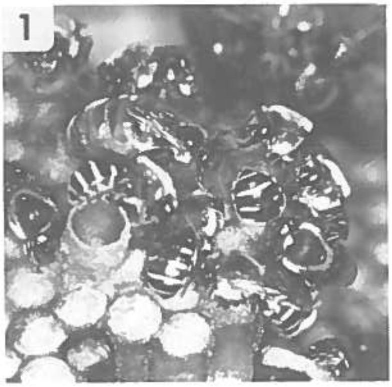
RESULTS

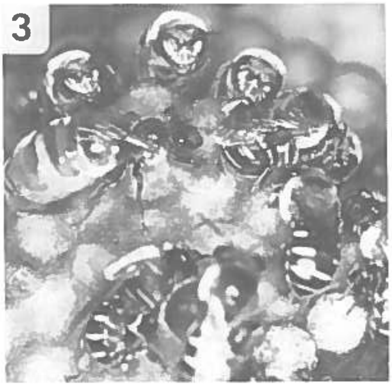
a. Description of characteristic behavioral elements of the queen

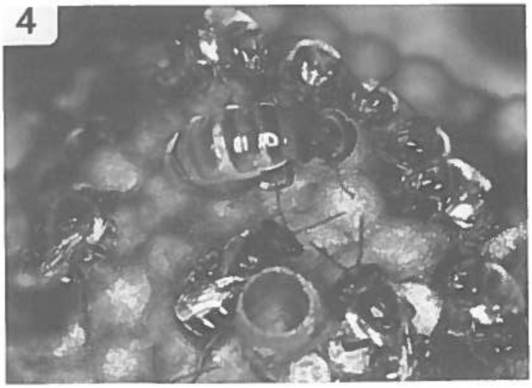
The cyclic activity of building, provisioning and oviposition is strongly reflected in the locomotive pattern of the queen. During the extra-oviposition periods, her behavior is characterized by long periods of akinesis ("resting"). Generally she remains at a fixed place or at one of several places nearby, under or partly under nest structures such as combs or food pots. From here she undertakes short excursions through the nest, after which she returns to the same spot. Both during resting and walking she performs a remarkable movement of the wings. This consists of an abrupt spreading of the wings up to an angle of 45^0 in a single beat without vibrations. Immediately thereafter the wings are brought back into the normal position on her abdomen. The duration of the total movement is less than half a second. Another remarkable behavior is the stroking of the abdomen by the hind legs. The abdomen is bent ventrally. The queen may stroke dorsal, lateral and occasionally ventral parts. The stroking is done with considerable force as can be noticed from the way in which the cuticula is pushed inwards by the stroking legs. A few times we noticed that the stroking queen lost her balance and she even fell from the comb.

In the interactions with workers the queen demonstrates other elements of her behavioral repertoire. Commonly she engages in mutual antennations with workers during which both parties touch each other on the face and head appendages. The queen also antennates other parts of the worker's bodies, but she herself is also commonly antennated all over her body by workers. Very obvious are the dashing advances that workers direct to the body of the queen with raised fore-body and opened mandibles. This typical worker movement also known as "darting", is common to most *Melipona* spp. (SAKAGAMI, MONTENEGRO and KERR, 1965). These typical worker behaviors will not be treated further here. The conspicuous interactions with workers that are commonly referred to as food solicitations (SAKAGAMI and ONIKI, 1963; SAKAGAMI, 1982) consist of intense buccal contacts between the queen and a worker. The sequential occurrence of this behavior is interesting. Workers standing in front of the queen and/or not avoiding her approach, are grasped firmly by the head. The queen holds the worker's head by her fore legs and touches this with her antennae and mouth parts. Apparently it is the response of the worker which determines how the interaction proceeds. Most commonly the worker remains crouched on the comb turning her head with the vertex towards the queen. The queen then holds on to the worker's head while intensively touching the worker's face and mouthparts with her antennae and front legs. Usually this grabbing takes less than 5 seconds, after which the worker is released. Another response of the worker is to lift her head a little and spread the mandibles. Subsequently the queen extends her glossa between the open mandibles and takes the food which may now be offered by the worker. This interaction generally takes 10-30 seconds, but it may last up to 2 minutes.

When the queen arrives at a completed brood cell where the surrounding workers are noticeably excited her movements become faster and the workers, which in turn insert their head or fore-bodies into the cell are tapped by the queen on the head and other dorsal body parts while inserting (Fig. 1). These tappings are very fast drumming movements by fore legs and antennae. They may continue even if the workers becomes partly hidden inside the cell. The tapping legs and antennae then drum on the dorsal part of the worker's abdomen. This behavior is most intense immediately prior to and during the first discharges of larval food by these workers. Within a short period a number of workers will empty their honey stomach into the cell with dis-







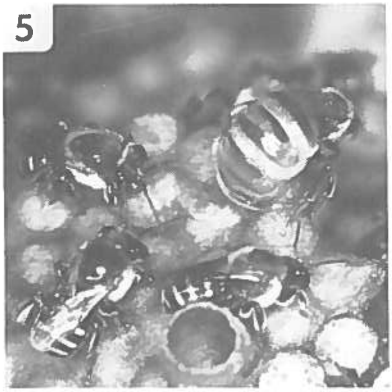


Fig. 1. Body-inserting worker is being drummed by the queen at the beginning of the food discharging sequence.
Fig. 2. Brood cell filled with larval food.
Fig. 3. Oviposition by a worker at the end of the food discharging sequence.
Fig. 4. Oophagy or uptake of larval food from the cell.
Fig. 5. Oviposition by the queen.

tinctive abdominal contractions (Fig. 2). The discharges follow one after another in quick succession. At this point the queen recedes a little from the cell, stepping back about one body distance towards the centre of the comb. Intermittently the queen displays some other behavioral elements. These are directed immediately towards the brood cell or its contents. She may step forward and insert her head and thorax partly into the cell, or she may put her head above the open cell and antennate the walls of the cell. This behavior is similar to the common "cell inspections" described for other species (SAKAGAMI, MONTENEGRO and KERR, 1965). Generally she performs a few of these inspections during each POP always keeping at a distance from the cell. When the workers have provisioned the cell the queen ingests food from the cell. This food may consist of a worker egg that has been laid at the end of the discharging period or it may be some of the larval food deposited previously by the workers. So far it has been impossible to establish whether the queen also ingests larval food whenever she devours a worker egg. In any case the queen always ingests some food, either egg or larval food, immediately prior to her ovipositing (Figs. 3 and 4).

During "fixation" and the discharging phase the queen always stands on the comb in a horizontal position. Only when the first cells of a new comb, that still have no neighbouring sealed cells are provisioned, will she hang vertically on the wall of these cells during the discharging phase. In order to mount the cell she steps forward without turning to the side. Consequently she arrives at her laying position with her head directed to the periphery (Fig. 5). After reaching this position she beats the wings several times. A few contractions of her abdomen indicate the actual laying of the egg.

b. Locomotion of the queen during the extra-oviposition period

The time spent by the queen on the comb and at her resting places was measured by continuously recording her behavior during a number of extra-oviposition periods. In addition, we recorded the time during which she was walking or resting. The results are presented in Table 1. It is evident that she stays mostly on the floor of the hive. In six recordings of extra-oviposition periods with an average duration of 8250 seconds, she remains resting on the floor of the hive for 74.2% of the time. It was also apparent that she remained immobile for most of the observation time (on the average 77.8%). The time spent on the comb has on average only 18%. In Figs. 6A and

Colony	Date	duration extra-oviposition period(sec)	duration observation time (sec)	queen visible (sec = 100%)	Percentage of time							
					ON FLOOR			ON COMB			WALKING	SITTING
					walking	sitting	total	walking	sitting	total		
F2	10-4	5100	4800	3036	20.6	55.5	76.0	22.3	1.6	24.0	42.9	57.1
F1**	21-6	12000	12000	10557	6.3	80.5	86.8	5.3	7.9	13.2	11.7	88.3
F1**	5-7	5400	5400	4325	3.7	63.1	66.9	22.8	4.4	33.2	32.5	67.5
F1**	5-7	7200	6600	6531	5.4	87.2	92.5	7.3	0.2	7.5	12.7	87.3
F1**	11-7	7800	7800	7441	4.8	76.1	80.9	12.0	7.1	19.1	16.8	83.2
F1**	13-7	12000	11700	9998	6.3	82.6	88.9	10.1	1.0	11.1	16.4	83.6
$\bar{x}$		8250			7.9	74.2	82.0	14.3	3.7	18.0	22.2	77.8

Table 1. Activity of the queen of *M. favosa*: time spent walking and sitting on comb and floor of the hive during the extra-oviposition period (EOP). One EOP was recorded in colony F2; the others in colony F1**.

fig.6a

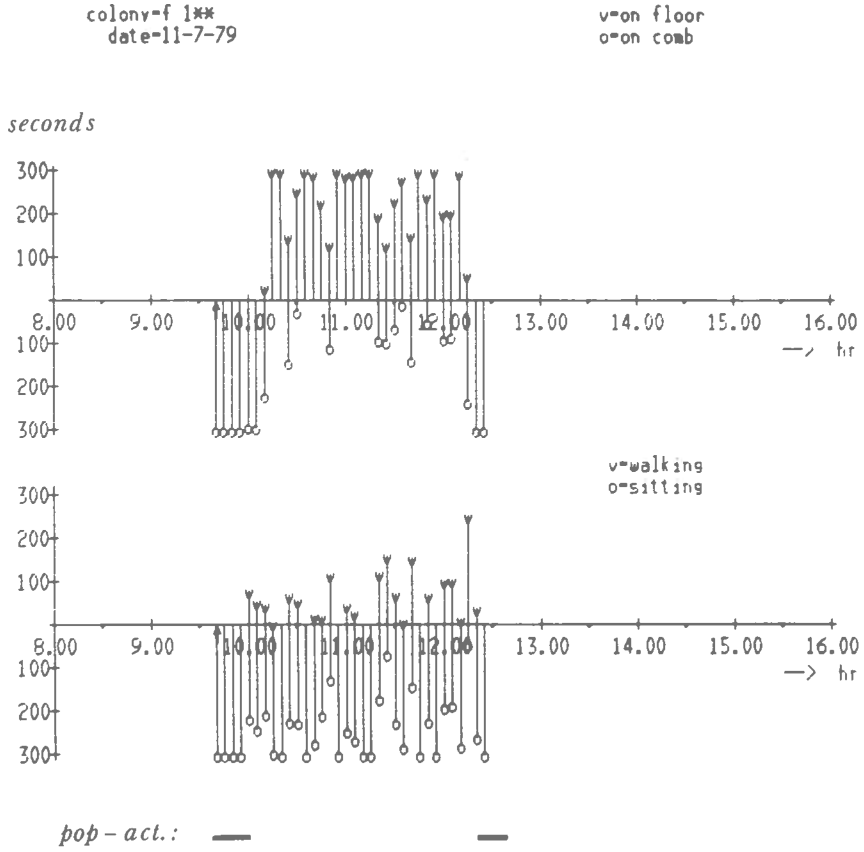


Fig. 6. Two cases as examples of the locomotive pattern of the queen. Observations were carried out from the first until the last POP indicated.

Fig. 6a. A typical example of the temporal structure of the locomotive pattern of the queen during a representative extra-oviposition period and the two neighbouring oviposition periods. The one histogram shows for every successive observation period of 5 minutes (300 seconds) how long she was either on the comb or on the floor of the hive. The other histogram shows how long she was walking or sitting. The discrepancy of the total indicated time indicates for each block of 5 minutes the time that the queen was invisible.

fig. 6b

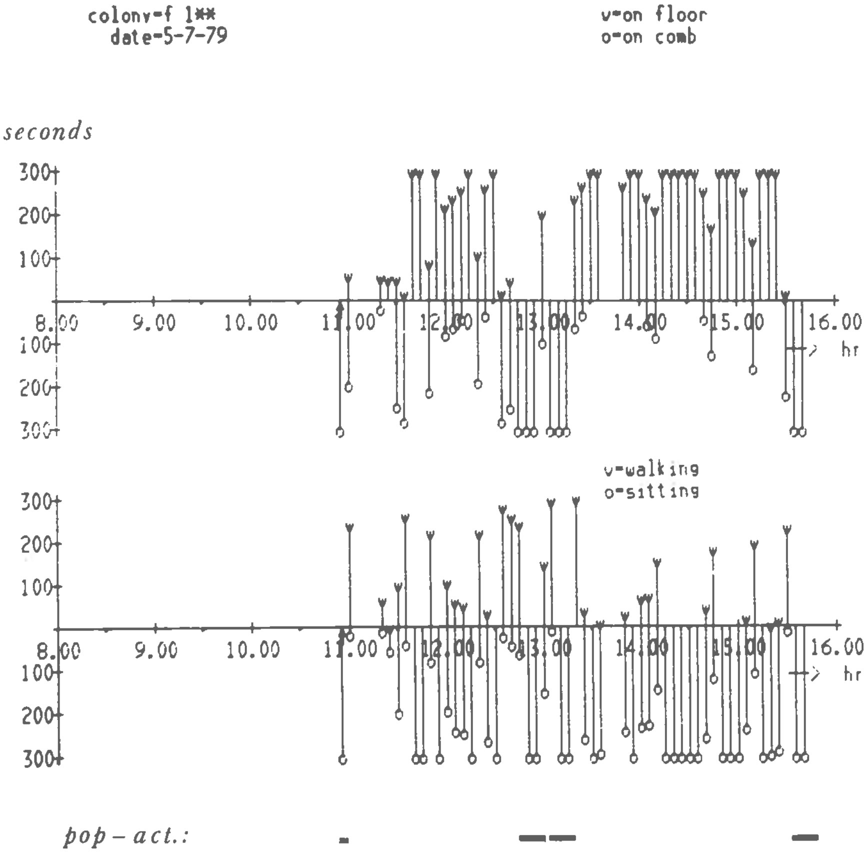


Fig. 6b. Represents 2 extra-oviposition periods that were separated in an unusual way by two POP's that followed each other nearby immediately. During the first extra-oviposition period the queen was walking more and was present longer on the comb than we usually observed. In the second extra-oviposition period the locomotive pattern of the queen was very similar to the typical pattern represented by the case of Fig. 6a. (see discussion).

6B we have illustrated two examples of the temporal structure of the locomotive pattern of the queen, together covering three extra-oviposition periods and the neighbouring oviposition periods. These illustrations show during which part of each 5 min. period the queen was walking or sitting. In the same way is shown whether she was on the floor or on the comb. These examples are representative for the cases studied. They clearly show the general locomotive pattern of the queen behavior mentioned above. The two successively recorded extra-oviposition periods of colony F 1** on June 5th represent a further interesting aspect (Fig. 6B, see also Table 1). The first extra-oviposition period illustrated in this figure is not so markedly characterized by the predominant "resting away from the comb" by the queen, as explained above and as indicated by some other results. At the end of this particular extra-oviposition period two cells were ready at the same time and both were provisioned and oviposited very shortly after each other. This is unusual for *M. favosa.*

c. Wing beats and stroking behavior

The occurrence of the beating of the wings and the stroking of the hindlegs along the abdomen was measured during 5 continuous observation series of the extra-oviposition period. The temporal distribution of these behavioral elements of the queen during POP was further recorded in 41 POP sequences. During the extra-oviposition period both the wing beats and the strokings with the hind leg were executed with a rather variable frequency. The average results are presented in the Tables 2, 3 and 4. Also during the POP the frequency of the beating of the wings did not change notably. However, the stroking frequency was observed to increase during POP (Table 4). This was particularly the case shortly after the beginning of the food discharges by the workers. The queen of colony F 2 performed the wing beats with a notably higher frequency (Table 2). This may be related to the fact that this queen was still very young. She emerged in this colony and had been accepted only two weeks before as the new queen of the previously queenless colony and she had been laying for only one week before these observations were carried out. We started this queenless colony by separating some brood combs and adults from another nest when it was transferred from its natural tree trunk to the observation hive. From the moment of the queen's acceptance by the colony till about the first days she was ovipositing, she even carried out her

Colony	Date	Number of observation periods of 5 min.	Number of POP's in this period	Frequency per period of 5 min.			
				WINGBEAT		STROKING	
				$\bar{x}$	SD	$\bar{x}$	SD
F2	10-4	41	2	12.2	9.6	3.7	2.9
F1**	21-6	62	2	0.6	0.9	2.2	2.6
F1**	5-7	53	4	0.3	0.6	2.8	2.9
F1**	11-7	34	3	0.4	0.7	3.4	3.1
F1**	13_7	42	2	0.5	0.9	2.7	3.3

Table 2. Activity of the queen of M. favosa: Average wingbeat and stroking behavior during 6 observation series. Each series included at least one EOP.

	Freq. per min. during different parts of the time from first discharge until oviposition (D-period)									
Colony	average number during D-period		during 2 min. before first discharge		total D-period		first half		second half	
	$\bar{x}$	SD(n)	$\bar{x}$	SD(n)	$\bar{x}$	SD(n)	$\bar{x}$	SD(n)	$\bar{x}$	SD(n)
A	1.0	1.2(4)	1.3	0.8(4)	0.2	0.2(4)	0.3	0.3(4)	0.1	0.1(4)
B	10.2	8.2(23)	1.8	1.6(14)	1.2	0.9(23)	1.6	1.2(23)	0.8	0.7(23)
C	7.8	5.7(8)	0.7	1.4(5)	1.2	0.7(8)	1.1	0.8(8)	1.3	1.1(8)
D	8.8	8.2(6)	6.0	2.4(5)	1.3	0.6(6)	1.9	1.0(6)	0.8	0.6(6)

Table 3. Activity of the queen of M. favosa: Wingbeat behavior during POP in the colonies A, B, C and D.

			Freq. per min. during different parts of the period from first discharge until oviposition (D-period)							
Colony	average number during D-period		before first discharge		total D-period		first half		second half	
	$\bar{x}$	SD(n)	$\bar{x}$	SD(n)	$\bar{x}$	SD(n)	$\bar{x}$	SD(n)	$\bar{x}$	SD(n)
A	6.0	2.3(4)	0.1	0.1(4)	1.6	0.7(4)	1.6	0.8(4)	1.6	1.2(4)
B	8.4	6.7(23)	0.3	0.6(15)	1.1	0.8(23)	1.5	1.1(23)	0.8	0.7(23)
C	14.1	8.9(8)	0.4	0.4(7)	2.2	0.9(8)	3.1	1.3(8)	1.3	1.0(8)
D	15.5	15.8(6)	0.2	0.2(6)	2.2	0.6(6)	3.1	1.1(6)	1.4	0.4(6)

Table 4. Activity of the queen of *M. favosa*: Stroking behavior during POP in the colonies A, B, C and D.

strokings with her two hind legs at the same time, first drawing them over the abdomen, then over her thorax and the head. This queen was very mobile. From the moment she stopped her excited walking she was immediately surrounded by a relative large court of workers.

d. Graspings and food rewards

We recorded the trophallactic activity in a specific observation series of six different days during each of which we observed continuously for 3-4 hours. This included five POP sequences. We recorded 509 food solicitations during a total of 862 minutes of observation, including 59 minutes of POP activity. Only nine (1.8%) of these solicitations were rewarded with a food donation. There were no food solicitations during the POP sequences. The absence of food solicitation during POP sequences was also found in numerous other observations. It is of interest to note that the frequency of food solicitation increases during the progress of the extra-oviposition period and further that the rewarded solicitations occur generally just before the start of the POP.

We also studied the trophallactic behavior of the queen in colonies with individually marked bees. These observations were undertaken in order to study the behavior of the workers just before and during POP and therefore they did not cover complete extra-oviposition periods. As a consequence we observed a higher percentage of rewarded solicitations here. Out of 160 solicitations six (3.8%) were now rewarded. Shortly afterwards these donating workers played an important role in the next POP. Five of these six donor bees started the provisioning of the next brood cell by performing the first discharge of larval food.

DISCUSSION

Our study reveals the pattern of activity of the queen in a colony of *M. favosa*. During most of the time she is almost completely inactive. Only around the time that ready cells are provisioned and oviposited does she become more active and walks through the nest. We have suggested that this change is brought about by the court workers (SOMMEIJER, 1982). In studying court behavior during the extra-oviposition period, we found that 86% of all court acts were initiated by workers who played important roles in the construction and provisioning of the brood cell that was most advanced at that time. Brood cells are built successively and oviposited one at a time in *M. favosa*, as in most other species of this genus (see: SAKAGAMI and ZUCCHI, 1974). There is generally a longish extra-oviposition period between the POP's of two successive brood cells. In relation to the assumed regulatory effect of court behavior in the extra-oviposition period it is of special interest to note that the first extra-oviposition period of colony F 1** which was recorded on 5-7-79, represents unusually high activity on the part of the queen. It is suggested that this is related to the fact that during this particular extra-oviposition period the building of two brood cells was completed. As can be seen from Fig. 6B, this extra-oviposition period is concluded with the oviposition in 2 cells in succession. The number of court bees that were active in stimulating the queen during this extra-oviposition period may have been larger than normally observed by us.

In many species of social insects the queen receives her food partly or wholly from her workers directly. Such trophallaxis is often preceded by typical begging behavior on the part of the queen, consisting of antennal touchings and front-leg tappings (MONTAGNER, 1967; BONAVITA-CONGOURDAN, 1982). In *M. favosa* the reward ratio of this behavior is very low: the queen receives food only rarely in this direct way. The same has been noted by SAKAGAMI and ZUCCHI (1968) in other stingless bees; in earlier studies they even failed to observe this behavior. This fact and the comparatively exaggerated nature of soliciting behavior leads to the assumption that this has evolved into a secondary function: By means of this ritualized soliciting the queen maintains her dominance over the workers. Such dominance behavior is well known from the queens of various social wasps (PARDI, 1948; EBERHARD, 1969). For other *Melipona* spp. also it has been supposed that the trophallactic interactions of the queen with the workers have a ritualized character. In *Plebeia* and *Friesella*, taxa that are considered to be related to *Melipona* but to be phyllogenetically less specialized (SAKAGAMI, pers. comm.), these interactions are still considered to contain an aggressive component (SAKAGAMI et al., 1977). For this reason, SAKAGAMI, CAMILO and ZUCCHI (1973) consider that in *Melipona* the queen's grasping and touching of workers crouching in front of her as "dominating behavior". A possible analogy for the ritualization of trophallactic behavior in *Apis mellifera* has been described by KORST and VELTHUIS (1982). They found that less than 5% of the trophallactic interactions between workers of this species actually concerned transfers of food. They conclude that most trophallactic contacts in their experimental groups of bees served principally to communicate dominance status. We should stress the resemblance between the behaviors of the queen while grasping, anywhere in the nest, workers standing in front of her, and while touching workers who insert their bodies into the ready brood cell at the start of the larval food regurgitations. Graspings occurring immediately prior to discharges of larval food pass smoothly into the typical dorsal drummings on body-inserting workers. The queen always feeds on either larval food or on eggs deposited by the workers, immediately prior to ovipositing herself. In this way the queen receives her major food supply indirectly from the workers.

It is evident that for the synchronization and coordination of activities of both queen and workers in connection with the oviposition process, specific communication mechanisms must have evolved. The function of the conspicuous movements with wings and hind legs has still not been satisfactorily clarified. We assume that these behaviors are related to the distribution of queen pheromones. Pheromonal substance may be spread over the body of the queen and be further spread by the wing movements. Our observations on the establishing of dominance by the young queen support this assumption. CRUZ-LANDIM (1980) reported the existence of specific queen-pheromone glands in *Melipona quadrifasciata*. These glands, not occurring in workers, are located in the tergites II, IV, V and VI. After the first report of the existence of well-developed scent glands in queens of stingless bees by CRUZ-LANDIM (1962), KERR (1969) assumed that these glands might have an important role in the production of sex attractants. KERR refers also to the finding by CRUZ-LANDIM of "gland-waxlike structures within the mandible and in the tarsi". IMPERATRIZ-FONSECA (1977, 1978) refers also to the supposed importance of pheromones secreted from the tergites and their possible spread over the queen's body by means of leg strokings during interactions between an old and a virgin queen of *Paratrigona subnuda*. She also noticed workers of *P. subnuda* licking the tergites of young queens. The incidence of chemical communication for queen recognition is evident from these observations. Further research is necessary to clarify the causal mechanism of this communication channel.

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IV SOCIAL BEHAVIOR OF STINGLESS BEES: "BEE-DANCES" BY WORKERS OF THE ROYAL COURT AND THE RHYTHMICITY OF BROOD CELL PROVISIONING AND OVIPOSITION BEHAVIOR

M.J. Sommeijer and L.L.M. de Bruijn

SUMMARY

In *Melipona favosa* the court of workers surrounding the queen during the extra-oviposition period is formed principally by those workers who are actively participating in cell building in that period. These bees shuttle intensively between the cells, which they are building, and the distant resting queen. It is concluded that the gradual increase in court acts performed by these workers is of importance for the regulation of the locomotive pattern of the queen. The principal builders are the most active court bees at that time. The specific behavior of the court workers should be considered as a form of "competitive communication". Similar ritualized court activity has been described for other stingless bee species. In certain species very striking worker-queen co-actions, termed by us as "bee-dances", have evolved. It is concluded that these "bee-dances" have developed for the purpose of conveying information to the queen and that this evolution is, in this group of bees, related to the peculiar temporal structure of oviposition.

INTRODUCTION

This paper deals with the behavior of worker bees in the court around the queen and the relation of this court activity to the typical oviposition in colonies of stingless bees.

The unique social phenomenon of mass-provisioning and subsequent ovipositioning of brood-cells by stingless bees ("provisioning and oviposition process, POP", SAKAGAMI et al., 1965) forms the basis of their typical intranidal behavioral periodicity. This rhythmicity consists of the regular alternation of the "extra-oviposition period" with the "oviposition period". The extra-oviposition period is subdivided into the consecutive "quiescent and transcient phases", and the oviposition period in three successive "patrolling, arousal and oviposition phases" (SAKAGAMI et al., 1974). The succession of these phases of intranidal reproductive behavior is schematized in Fig. 1, taken from SAKAGAMI et al., 1974.

This typical cyclic behavioral pattern includes several activities of the workers such as alternate body insertions in the cell, cell provisioning, facultative worker ovipositions and cell operculation in the oviposition period. These activities are alternated with less "excited" cell construction activity during the extra oviposition period. Such "rhythmic" ebbs and flows of "oviposition excitement" (SAKAGAMI et al., 1974) are a striking feature of stingless bee behavior. The rhythmicity is most obvious in species where the construction of a group of cells is synchronously started after the previous oviposition and where the ovipositions are batched.

The basic pattern of this cyclic behavior pattern can be observed even more clearly when the behavior of the queen is taken as a focus. During most of the extra-oviposition period the queen stays away from the area of the brood-nest where cells are under construction (SAKAGAMI et al., 1974; SOMMEIJER, 1981; SOMMEIJER et al., 1982). During most of the oviposition period the queen is on the comb. She is then involved in interactions with workers who start the cell provisioning soon after her arrival at the comb. The queen oviposits on top of the larval food after a cell has been provisioned. She then withdraws again from this area of the brood nest. According to the species, a worker oviposition may occur before the queen oviposits, which at this stage is followed by queen oophagy, immediately prior to the queen's oviposition.

There is no information available about the regulation of this rhythmic oviposition behavior. In this respect we were interested by an observation made during one of our previous studies on worker behavior. For these observations we had marked individually all workers of a colony of *Melipona favosa*. In these observations it occurred to us that some of the workers who participated in the building of a brood-cell were also attending the royal court around the queen at her resting place away from the youngest comb ("advancing edge"; MICHENER, 1974) during the extra oviposition period, preceding a particular POP. We considered this of particular interest, especially since we had found earlier that the bees who participated the most actively in the provisioning of broodcells had distinguished themselves as

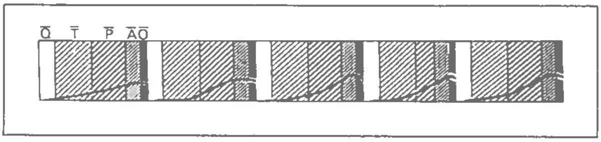

Fig. 1. The succession of the typical phases* of intranidal reproductive behavior and the rhythmic ebbs and flows of oviposition excitement (from Sakagami et al., 1974). The rhythmicity is most obvious in species with synchronous cell building. EXTRA OVIPOSITION PERIOD consisting of: Q. *quiescent phase**, comb surface is completely free from oviposition excitement. Time since the end of the previous oviposition to the revival of some primordia of oviposition excitement, such as the first visit of the queen with the comb in types where cells are built successively, or the beginning of cell construction in species where groups of cells are built synchronously; T. *transient phase**, from the end of Q, to the beginning of P. OVIPOSITION PERIOD consisting of: P. *patrolling phase**, increasing visits of queen with, and prolonged stay by her on the comb, after the appearance of completed cells with collars and the congestion of workers at cells with frenetic body movements, usually accompanied by alternate insertions of fore-bodies into the cells; A. *arousal phase**, the period between the final arrival at the comb of the queen and the beginning of the first oviposition process; O. *oviposition phase**, the complete sequence of cell provisioning, facultative worker ovipositions, uptake of food by the queen (egg or larval food), queen ovipositions and operculation.

*Sakagami recently replaced the term "phase" by the term "stage", as "phase" is now used for lower units within the actual oviposition process (Sakagami et al., in press).

the major constructors of this cell (SOMMEIJER et al., 1982). We also found that the typical locomotive activity pattern of the queen in thriving colonies of *Melipona favosa* implies that she arrives at the comb just at the right time, when the most advanced cell is ready for the start of the oviposition process (SOMMEIJER, 1981). These results together with the preliminary observation lead us to formulate a hypothesis concerning the identity of the court workers. We hypothesize that the court is specifically formed by brood cell constructing workers and that the court behavior of these workers might be of importance for the regulation of queen behavior e.g. stimulate her departure from her resting place to the advancing edge.

In order to obtain further insight into the function of the royal court in stingless bees we have studied some aspects of this behavior in *Melipona favosa*. In this paper we report on the relation between worker participation in court behavior and their POP activities in this species. Finally, we will discuss some other available data on court behavior in stingless bees, in connection with our interpretation of the function and evolution of this behavior.

MATERIAL AND METHODS

The observations were carried out in 1979 on a colony of *Melipona favosa* in Curepe, Trinidad, West-Indies. The colony was housed in an observation hive with free exit to the outside. All workers of this colony were individually marked upon emergence. Observations on the behavior of workers and queen were carried out by using red light. The interactions of individual workers with the queen were recorded. The behavioral elements and interactions recorded away from the comb were:

- antennation of the body of the queen by a worker;
- mutual antennation of worker and queen;
- "darting", a dashing advance of a worker towards the queen, immediately followed by a retreat (cf. SAKAGAMI et al., 1965; SOMMEIJER, 1981);
- "grasping", the queen approaches a worker, grabs both sides of the worker's head with her front legs and antennae. The worker responds by crouching, turning her vertex to the queen (this form of queen behavior is commonly referred to in literature as "food-begging");
- "queen-feeding", the same initial action of the queen as "grasping" but now

the worker responds differently by offering food to the queen.

On the comb we recorded the cell-directed activities of the workers. During the extra-oviposition period we recorded:

- cell-building activity

During the oviposition period we recorded especially the:

- food discharges

However, we also recorded miscellaneous POP activities such as:

- head insertions
- body insertions
- tappings that the workers receive from the queen while inserting into the cell (cf. SAKAGAMI et al., 1965; SOMMEIJER, 1981).

We recorded the identity of the court workers, their individual participation in this behavior and the individual participation in cell building during the same extra-oviposition period. In the oviposition period we recorded the individual participation in cell-provisioning and in other activities. Observation periods for the recording of court activity and cell construction covered complete extra-oviposition periods. Therefore they were started and terminated at the occurrence of POP sequences. On the first observation day five minutes of recording court behavior was alternated with five minutes recording of building behavior. However, it turned out that during the successive cell building observation periods, the same individuals were generally encountered as constructors. Therefore, the observation schedule was changed for the following observation days into a scheme in which periods of 30 minutes observation of court behavior were alternated with periods of 5 minutes observation of cell building. This series of observations was carried out on 5 days in 1979 (31-7; 1-8; 3-8; 5-8; 6-8). The daily observation was on the average 4 hours. In addition to this series where we specifically followed the courting workers, we had also recorded the interactions of workers with the queen while the queen was the focal animal (on the days: 21-6; 5-7; 11-7; 13-7). In this earlier series of observations the queen also frequently contacted the same workers in the court as during the following provisioning and oviposition phase. Of course, this type of observation does not provide information on the cell building activity of these workers. However, during the POP these workers were seen to discharge and/or to show head and body insertions into the cell. Like the food discharges, these insertions are strongly correlated with building behavior, as was confirmed earlier (SOMMEIJER et al., 1982). Therefore we feel that these data should be included in our present analysis.

RESULTS

Behavior of workers and queen during the existence of the royal court

During most of the extra-oviposition periods the queen sits quietly at one of her "resting places", away from the cells that are being built. If she remains undisturbed, her quiescence may continue for quite a long period. However, soon after the queen has settled down a number of workers (1-6 individuals) may surround her. The workers keep at "bee-body" distance from the queen, facing her from all sides. Such workers often show distinct alternating body movements towards and away from the queen without shifting a leg. During their movement towards the queen the antennae are stretched and may touch the queen's body. While the workers retreat, their antennae and head are lowered. This touching of the queen by the workers may be performed about 20 times per minute, and can be directed at all parts of the queen's body, but is generally directed at the head and the abdomen. Figures 4c and 4d show examples of court positions. Licking of the queen, which commonly occurs in the court of the honeybee, *Apis mellifera*, was never observed. After a few minutes an individual worker will leave the royal court and may be replaced by another worker. This results in a fluctuation in the numbers and individuals involved in court behavior at any given time.

The queen may react to the antennal touching of court workers by turning towards them, after which a mutual worker-queen antennation often takes place. Such a mutual antennation may result in:

I a fast retreat of the worker in a backward walking movement

II "grasping" by the queen

III "darting" by the worker towards the queen, immediately followed by a quick retreat.

When grasped by the queen, the worker can respond in two different ways. She may either offer food to the queen or may respond by crouching. In the latter case there is often a series of graspings, in between which the worker retreats a short distance. Especially after mutual antennation and grasping, the queen may follow the retreating worker in this way for a distance of up to several cm. After that she may come to rest in a near-by new spot or return to her old place. When walking on the floor of the hive the queen does not have continuous contact with workers. Workers do not follow the walking queen and the contacts are brief since the workers she encounters usually retreat very fast. When the queen walks on the combs and "waits" by the cell the above described behavioral elements occur more frequently again. Quick-acting workers surrounding the ready cell may now turn to the queen, even if she is still a few cm away from the cell, and touch her with their antennae.

Relation between court activity and activity directed to the brood cell

During the 9 observation days an average of 47 individuals were seen to participate at least once a day in the royal court. Of these, 48% participated in building and/or provisioning ("BP") on the same day; these bees were responsible for 86% of all observed court acts (n=4053) directed at the queen when in the court. It turned out that 25.2% of the observed court-bees were recorded as "court bee" on the basis of only one single interaction with the queen during the observation of that day. We know the activities for about 50% of these bees at the different days of age. This reveals that on the day these bees were recorded in the court, they were actively participating in the manipulation of waste at the rubbish dump in the colony. Bees involved in this activity frequently cruise over the floor of the hive on their way to and from this dump, which enhances their chances of casual interactions with the queen. The court activity of the BP court bees (with behavior directed towards the cell on the same day as court behavior) was different from that of the bees observed in the court but not working on cells. The differential court activity of the two groups of court bees is illustrated in Fig. 2. In this figure the degree to which single bees took part in court activity is indicated separately for BP and non-BP bees by classifying these individuals over a range of court-activity categories. The figure shows that a high level of court activity is performed exclusively by BP court bees. If we exclude the group of bees that only have one single interaction, the percentage of BP individuals in the court rises to 81%. They are responsible for 91% of the worker interactions in the court (Table 1). The BP bees showed a predominant participation in all the court activities (Table 2). This table also gives the total scores for the different behavioral elements in the court. The most frequent court act was the antennation of the queen's body (n=1982,

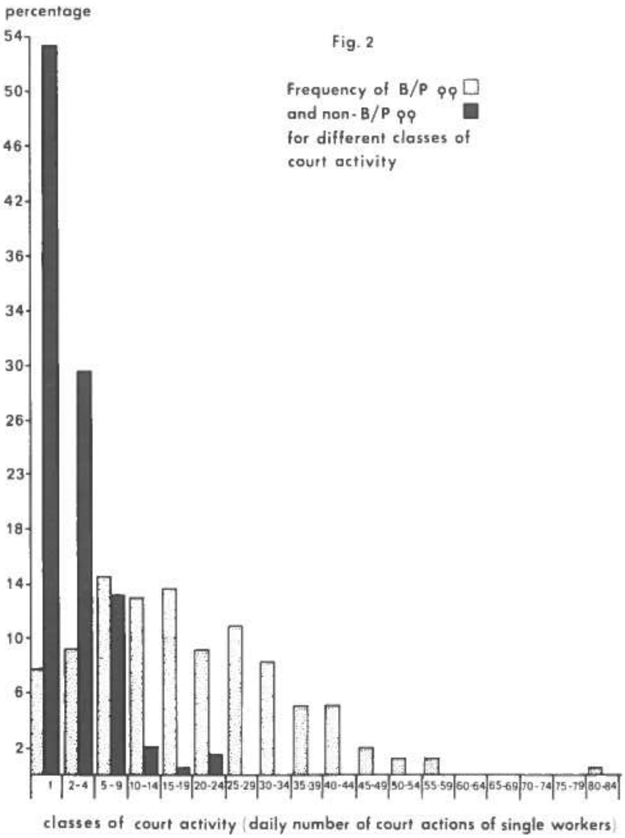

Fig. 2. The degree to which single bees took part in court activity is indicated separately for BP- and non-BP bees (see text). Individuals are classified (in percentages) over a range of court-activity categories. These categories indicate the number of daily court actions by single workers (for example: more than 50% of all recorded non-BP workers performed only 1 court act per day).

Table 1. Relative participation of BP bees in court on floor of hive.

A: of all court bees									
% of	data of observation								$\bar{x}$
	21/6	5/7	11/7	13/7	31/7	1/8	3/8	5/8	6/8	
bees	29	60	70	40	57	50	49	43	32	48
acts	79	94	84	78	92	90	89	85	75	85
B: of all bees with more than 1 court act										
bees	72	93	78	76	100	90	91	74	53	81
acts	87	98	86	84	100	97	96	92	79	91

The percentages represent the number of court acts and of bees in the court around the queen away from the comb, for building and/or provisioning (BP) bees. Table 1B represents exclusively the bees with more than 1 court act.

Table 2. Participation of BP and non-BP workers in the various court acts (48 % of the bees are BP-workers).

	b		a		d		p		v	
	n	%	n	%	n	%	n	%	n	%
Acts by BP workers	1892	86.2	1213	86.5	199	88.8	189	85.1	9	100
Acts by Non-BP workers	304	13.8	189	13.5	25	11.2	33	14.9	0	0

b- antennation by a worker on the body of the queen
a- mutual antennation of queen and worker
d- "darting" (see: Material and Methods)
p- "grasping" (see: Material and Methods)
v- "queen-feeding" (see: Material and Methods)

Table 3. Correlation at individual level in connection with the participation of BP bees in the various activities.
Correlation coefficients are listed; *= Sign. at p= 0.05

day	21/6	5/7	11/7	13/7	31/7	1/8	3/8	5/8	6/8	total
CA - B	--	--	--	--	0.6*	0.6*	0.2	0.2	0.2	0.3*
CA - D	0.0	0.4*	0.4*	0.0	0.4*	0.8*	0.7*	0.6*	0.4	0.5*
CA - mPOP	0.3	0.4*	0.0	0.1	0.7*	0.8*	0.8*	0.6*	0.5	0.5*
B - mPOP	--	--	--	--	0.9*	0.5*	0.6*	0.3	0.4	0.5*
D - mPOP	0.7*	0.4*	0.6*	0.6*	0.7*	0.8*	0.9*	0.9*	0.4	0.7*
n bees	15	29	25	19	40	31	31	20	12	

The relative performance of activities directed towards the cell is positively correlated at individual level with activity directed towards the queen.

CA, court activity (away from the comb!)
B, building on brood cells
D, discharging larval food
mPOP, miscellaneous "oviposition" activities, directed towards brood cell during POP (e.g. mere body insertions)

for BP bees). Queen feeding was recorded only 9 times and was carried out exclusively by the BP bees (Table 2). Now it is obvious that *the royal court around the queen in the extra-oviposition period is formed principally by the workers who also engage in cell building in that same period, and/or who discharge larval food in the subsequent POP.*

Continuous observation on behavior of particular workers

After establishing that active builders participate predominantly in the court around the distant queen, we attempted to record the continuous behavior of these workers. A small number of bees, observed in POP-activities, were observed continuously, as far as possible, during the following extra-oviposition and oviposition periods. The results obtained for these workers gave a convincing picture of the quick alternations of court activity and cell bulding. These workers often moved directly from the cell towards the resting place of the queen. In Fig. 3 the succession of activities by these bees is represented by the indication of the scores for their various activities in each successive observation period of five minutes. These four representative workers performed in 32, 86, 67, and 78% respectively of the blocks in which they showed court behavior, also cell construction activity. High scores for cell building are correlated with high scores for court activity around the queen within the same five minute period. The quick alternation of these activities occurs throughout the total extra-oviposition period. These bees spend most of their time on these activities. However, it is also common for the focal worker to spend most of a particular 5 minute observation period soliciting food from other workers. The scores for departures from, and arrivals at the comb pertain mostly to the shuttling between the queen and the cell.

Correlation of court behavior of workers and their participation in building and/or provisioning, at individual level

The individual performance of the various activities in the two behavioral categories (court behavior and BP activity) was analyzed (Table 3). The individual level of activities directed towards the brood cell was positively correlated with the performance of activities directed towards the queen. This means that the bees who participate the most actively as builders of a

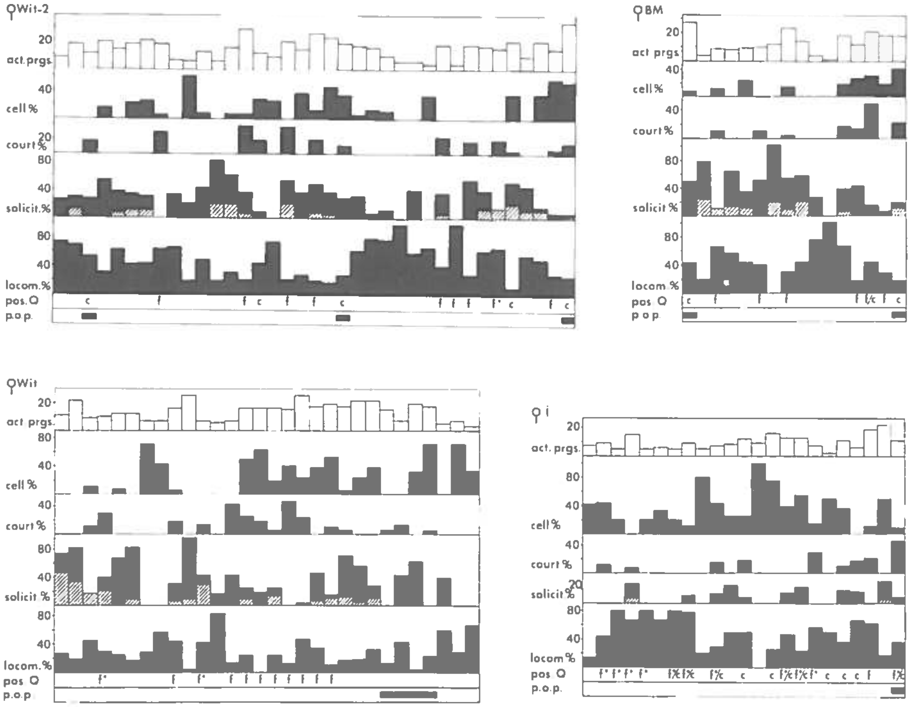

Fig. 3. The alternation of court and cell activity in a few continuously observed workers. Observed bees were participating in cell activities, e.g. building, nibbling on walls ("cell"), worker court activity ("court") and food solicitations directed at other workers ("solicit"). The number of rewarded solicitations was registered (▨). As a parameter for the locomotion of the worker ("locom.") we took the number of departures from and arrivals at the youngest comb, summed with the scores for disappearing under and appearing from covering nest structures. It appeared that building workers were thus lost from sight only when they moved towards the queen who in her turn was then invisible under nest structures. Consequently the quantitative performance of court acts by this worker could not be scored in these instances. The position of the queen during the 5-min. observation blocks, on the comb ("c") and on the floor ("f"), was not always registered. The occurrence of POP sequences is indicated ("P.O.P."). The total scores for all recorded activities is also indicated ("act. prgs."). The total observation time is divided into blocks of 5 minutes. The recorded activity is indicated as percentages of the total for every period of 5 min. separately.

certain cell who serve later on as the most active provisioners are the most active court bees during the extra-oviposition period (Table 3).

Gradual increase in court activity during the extra-oviposition period

Previously we found that the individual building activity of workers who subsequently become dischargers in the same cell increases according to the progress of the construction of the particular cell (SOMMEIJER et al., 1982). From preliminary observations we had obtained the impression that worker activity around the queen gradually intensified. Now we were interested in the progress of court activity during the extra-oviposition period. The temporal development of the royal court was therefore examined by dividing the total duration of each recorded extra-oviposition period into four equal parts. We analyzed the scores for worker court actions and for court individuals with regard to their progress over the four subsequent quarters in each of the recorded extra-oviposition periods (n=14). This revealed for the predominant BP bees a significant increase in both worker court actions (except for scores in quarter nr. 2 and quarter no. 3) and in the number of workers (Kendall's rank-correlation, $p<0.005$). This increase was not demonstrated by the non-BP court workers. Table 4 contains the averages for these 14 extra-oviposition periods. The occurrence of the various behavioral elements is indicated separately in percentages (see for the frequencies: Table 2). The infrequent queen feedings ("v") all took place in the last quarter.

DISCUSSION

The direct function of court behavior in M. favosa

During the extra-oviposition period the building bees shuttle frequently between the cells on the "advancing edge" and the queen who at that stage is at a considerable distance. Since the court acts are initiated by the courting workers, and since the occurrence of a court tends to be followed by locomotion of the queen, we conclude that the queen is brought to locomotion by these workers. This leads us to conclude that the court behavior of workers has communicative significance: workers attending the

Part of extra-oviposition period	• behavioral elements						number of workers
	b	a	d	p	v	total	
I	16	20	19	7		17	18
II	19	22	25	14		20	21
III	28	27	25	36		28	27
IV	37	31	31	43	100	35	37

Table 4. Performance of court acts by workers in the successive equal four parts of the extra-oviposition period (%).

court stimulate the queen to depart from her resting place so that she arrives at the completed cell at the proper time. It is evident that the performance of court behavior is quantitatively correlated at the individual level with building activity, and thus, as we found earlier, with cell provisioning (cf. SOMMEIJER et al., 1982).

The still evident aggressive nature of various court acts, e.g. "darting", is of particular interest with respect to the evolution of this communicative behavior. We can regard such specific court activity as the result of a ritualization of competitive behavior, which has led to specific communicative displays. This seems to be convergent with the evolution of dance displays in workers of *Metapolybia*. "Dancing" workers around the queen of this social wasp cause the queen to change caste and to start to work ("competitive communication", WEST-EBERHARD, 1982 and personal communication).

The gradual intensification of the royal court through the increase in the number of bees and in the number of court acts executed probably contributes in a direct way to the communicative value of court behavior. We observed that the stimulative effect of bringing the resting queen to locomotion is especially forceful when she is subjected to the simultaneous court activity of a number of bees (see also: Table 4 and Fig. 4c and d). When the queen receives antennation, dartings etc. from all sides, she turns continuously towards the bee who is touching her. In another study we specifically recorded the locomotive pattern of the queen (SOMMEIJER, 1981). We noticed that she was more active during the extra-oviposition period when 2 brood cells were at an equally advanced stage of building and were consequently provisioned and oviposited in quick succession. In this case the number of court bees was higher than normally observed.

It is important to note that the numbers of non-BP workers in the court did not increase nor did their number of court acts. The BP workers stayed longer in the court and did not avoid the queen at first encounter. Therefore they are more eligible for further, more intensive interactions such as grasping. Scores for food donations, which are performed exclusively by these bees, were always very low. However, this was consistent with all our other observations. The fact that the increase in court act frequencies is most noticable for grasping and queen feeding may have a specific value.

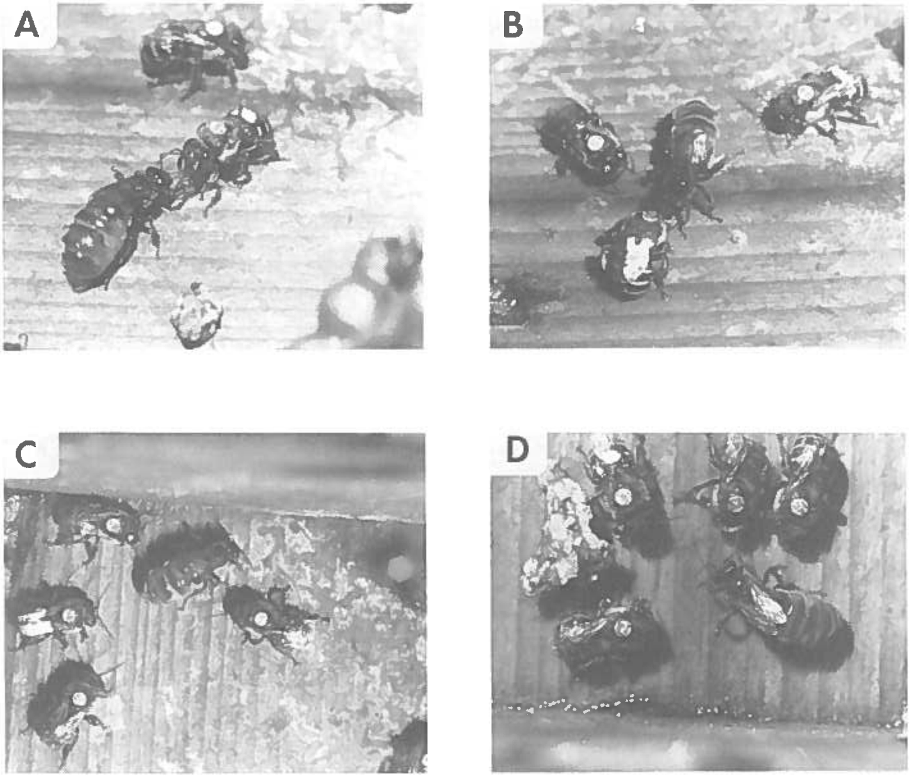

Fig. 4. Court activity in *M. favosa* (individually marked workers around the queen at her resting place). A. mutual antennation between queen and worker; B. a worker is being grasped by the queen; C. and D. a court of 4 workers around the queen. The queen turns towards the worker who directs an activity towards her (D).

The function of court behavior; a comparative approach

Descriptions of queen-worker co-actions and court behavior in various other species are consistent with our interpretation of the function of this behavior for the group of stingless bees in general. From the results of SAKAGAMI and ZUCCHI it appears that in most species the court around the queen is formed gradually when she is resting during the extra-oviposition period. A number of reported behavioral peculiarities of certain species become very interesting with respect to the assumed function of court behavior and in fact these offer additional support for our hypothesis. We will mention some of them. For the court in a weak colony of *M. compressipes* it is reported that the court never consisted of more than six bees (SAKAGAMI & ONIKI, 1963). These authors assume in this early publication that the small court will "mostly be conditioned by the weak colony size". The small colony size will, in our opinion, certainly have been the factor responsible; but in the following way. A small colony contains a small number of bees involved in building and provisioning at a certain time. The court around the resting queen is, according to the function that we are now proposing, restricted mainly to these initiating bees. The court in *M. favosa* is always of a small size.

Other descriptions indicate that the dashing movements called "darting" are carried out mainly by the workers standing in front of the queen. These are also the workers who most conspicuously perform the rhythmical movements, such as rapid advances and retreats common in most species. It is clear from several of these descriptions that these worker actions are initiating worker-queen co-actions. For example, SAKAGAMI & ZUCCHI (1974) describe such co-actions in *Hypotrigona (Trigonisca) duckei*: "Workers shake their antennae against the queen, but seldom touch her body except for the individual standing in front of the queen, who exchanges the antennal contact with her, the queen touching rather slowly, the worker more vividly. Workers repeat a mild pushing towards the queen, which is soon followed by a retreat. When the queen begins to proceed slowly, the worker in front gently retreats, or occasionally escapes without exaggerated manner. Or, the worker crouches against the approaching queen, lowering her head. Occasionally a mild darting with the mandibles opened appears after crouching but no severe ritualized aggressivenes is demonstrated". Following our own observations, we consider the communicative nature of this type of worker behavior as evident. The detailed and careful descriptions by SAKAGAMI and ZUCCHI of other specific forms of ritualized behavior of workers in queen-worker co-actions such as: "hypnotic turning" (cf. SAKAGAMI et al., 1973) further support our view (Fig. 5). A very striking form of worker behavior in this respect is described and illustrated by SAKAGAMI for *Meliponula bocandei*. The described "turning against the queen" (Fig. 6) means that workers in the royal court perform typical forward and backward movements in front of the queen, and after repeated darting movements directed frontally to the queen, they engage in a ritualized turning in front of the queen. This sequence of repeated dartings and frontal turnings is repeated many times, after which the worker again performs forward and backward movements or departs from the court. This typical court behavior, again carried out by only a limited number of workers at a given time, takes place exclusively away from the cell to be provisioned, when a court is gradually being formed while the queen is having a prolonged rest (SAKAGAMI et al., 1977). We consider these specific behavioral features of the various species as specific variations of court behavior, which in most species will have the same direct function: to stimulate the queen to move from her resting place so that she will set out on her way to the completed cells. It is not surprising that this relation had not been found earlier in other species. The technique of marking all bees of the hive without disturbance of the general intranidal behavior has not been used before. However, IMPERATRIZ-FONSECA (1977) in her study of the behavior of virgin queens in colonies with marked bees of *Paratrigona subnuda*, observed that "bees that were building broodcells were also courting the q (=physogastric queen) of the colony. Some of them were layers and they put some larval food into the brood cells and operculate them". From this description of the behavior of bees of a rather unrelated species we notice that in other stingless bees too the royal court is formed principally by building and provisioning workers. This is further support for the general application of our interpretation of this behavior.

The communicative nature of worker behavior with respect to the arrival of the queen on the comb and the start of POP has already been suggested by KERR (1969). While he refers to the direct function of "prefixation behavior" by workers at the cell, he comments as follows on the behavior of the excited workers around the structurally ready cell: "Then the workers become very excited, thus informing that "the cell is ready"; this "prefixation phase" lasts 22.27 min. on the average. Next the queen comes upon the comb and

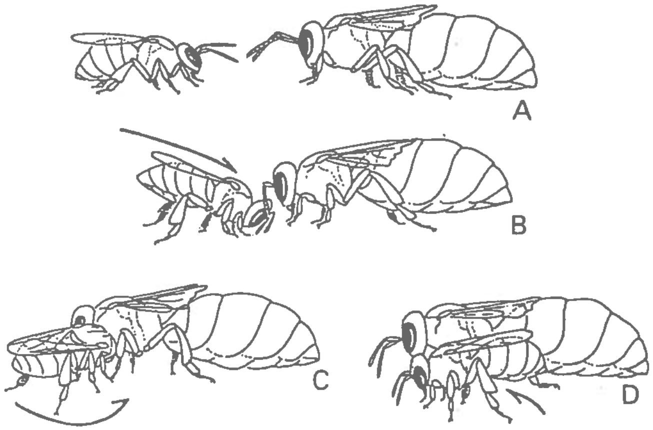

Fig. 5. "Hypnotic turning" in Plebeia droryana. Hypnotic turning is a peculiar response by the worker to the queen in Plebeia droryana (A) encounter; (B) worker darts at the queen with her head lowered; (C) worker slightly inclines the body and turns very slowly towards the queen; (D) sitting beside the queen and inclining the body, the worker slowly edges herself towards the queen (Sakagami, 1982).

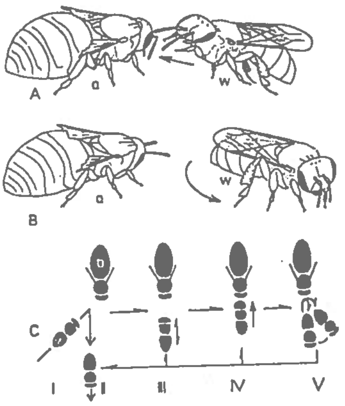

Fig. 6. "Turning against" in Meliponula bocandei. Behavior in a queen (Q) and worker (W) encounter. A. Darting by worker to queen. B. "Turning against" by worker. C. Sequence of various responses by worker. I= Arrival of worker. II= Avoidance. III= Repeated forward and backward movements. IV= Darting. V= "Turning against" (Thick arrows= Direction of behavior. Thin arrow= Sequence) (Sakagami et al., 1977).

examines the empty cell, ...". SAKAGAMI & ZUCCHI (1974), when receiving their own data on the temporal articulation of the oviposition process in various species, refer to the visits of the queen to the comb during the extra-oviposition period. They consider that the reciprocal worker responses stimulate the queen visits to the cell and lengthen her stay on the comb. However, when "A certain time after the previous IOP (Integrated Oviposition Process), either batched or not, the queen visits the comb where new cells are under construction....." they consider this appearance of the queen in general as ".... apparently driven from the revived laying motivation".

We conclude that the effect of the typical court behavior of stingless bee workers, including antennations of the body of the queen as well as elements with more obvious "aggressive" components such as "darting", is to stimulate the queen to move from her resting place and the encourage her to set out for the completed cells. We further suggest that several specific behavioral elements, described by other authors as typical forms of ritualized queen-worker co-actions, should also be interpreted from this point of view. We consider these behavioral features as true "bee-dances". This specific communicative behavior has evolved for the purpose of conveying of information to the queen. This evolution is connected with the peculiar temporal structure of oviposition in stingless bees. Among the 300 or so species of the diverse group of stingless bees we may expect considerable diversity of this type of "competitive communication" (WEST-EBERHARD, pers. comm.). In some groups with huge colonies the queen has no fixed resting places. In other groups she spends most time on the youngest combs. In this respect it is of evolutionary interest that the species of the genus *Melipona* live in small colonies, despite the morphologically specialized condition of this genus (SAKAGAMI, pers. comm.; WILLE, 1983). It is not precluded that this small colony size is of a secondary nature, as may be the communicative value of "dances" by the court bees. This group of bees offers great scope for the study of the evolution of sociality.

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V MORPHOLOGICAL DIFFERENCES BETWEEN WORKER-LAID EGGS FROM A QUEENRIGHT COLONY AND A QUEENLESS COLONY OF *MELIPONA RUFIVENTRIS PARAENSIS* (HYMENOPTERA: APIDAE)

M.J. Sommeijer, M.R. Dohmen, and M.J.A.H. van Zeijl

ABSTRACT.— Worker eggs laid in a queenless colony of *Melipona rufiventris paraensis* were similar to eggs laid by queens. These worker eggs developed into males. The worker eggs laid in a queenright colony had an incomplete reticulate chorion pattern and micropyle. These queenright worker eggs, which had a high tendency to topple over on the surface of the liquid larval food in the brood cell, were always eaten by the queen.

Introduction

The stingless bees (Apidae, Meliponinae) from the tropics are, like the honeybees (Apidae, Apinae), higly social insects. Their social behavior is, however, rather different. One basic difference is the way in which they feed their larvae. Like in solitary and in primitive social bees, brood cells in stingless bee colonies are mass-provisioned before the egg-laying. The provisioning of these brood cells occurs in a peculiar and very characteristic behavioral sequence, in which a small number of workers and the queen cooperate. In quick succession, these workers regurgitate the contents of their honey stomach into the cell for its provisioning with a liquid mixture of honey, pollen and glandular secretions. It is filled to 2/3 of its capacity. The elongated egg is now deposited on the surface of this liquid, on which it typically stands erect. Only the base end of the egg is in contact with the liquid. The cell is sealed immediately after the oviposition, and after 5-7 days the egg hatches. The fact that workers in queenright colonies of stingless bees can lay eggs would appear to be in sharp contrast to the mechanism by which the reproductive dominance of honeybee queens is maintained. The worker eggs (WE) may serve as trophic worker eggs (TWE) that are eaten by the queen or give rise to males (reproductive worker eggs, RWE). Some authors report slight morphological differences in these two types of WE's.

Within the Meliponinae, comprising about 300 species subdivided over the two tribes, Trigonini and Meliponini, there is a considerable diversity of the phenomenon of worker oviposition (WO) behavior. It is generally accepted that this variation especially occurs among the Trigonini, although the data are few and mainly pertain to *Trigona (Scaptotrigona) postica*, which is common in southern Brasil. In this species and other Trigonini, typical TWE's, which are reported to be anucleate and of different form, occur next to RWE's. TWE's are often deposited on the cell margin or even outside the cell. In *Scaptotrigona postica* they are laid before the queen oviposits, while RWE's are here laid after the queen's oviposition and next to the queen's egg in the same cell (Beig, 1972). In the Meliponini (*Melipona* spp.) WE's are always laid in the same manner as the queen eggs (QE's), i.e. standing erect on the surface of the larval food. The WE's laid before the queen's oviposition are very common in this group; these eggs are always ingested by the queen. It is assumed but not well demonstrated, that in *Melipona* spp. WO also may occur after the queen has laid, just prior to operculation. These WE's would then just as in the Trigonini, result into males. That *Melipona* workers are able to produce males is evident from the occurrence of this form of reproduction in queenless colonies. Since *Melipona* WE's are all deposited in the same way and since it is assumed that they resemble QE's, it has generally been accepted that in contrast to the Trigonini, WE's of *Melipona* are all the same. So WE's laid before the queen oviposits (but these are always eaten by the queen) and those laid during cell closure, as well as those laid in queenless colonies have been assumed to be capable of producing males (Sakagami, 1982).

Experimental

We tried to study the fertility of WE's laid in a queenright colony of *M. rufiventris paraensis*. Although rearing meliponids from the egg stage is in general quite simple (one has only to isolate the sealed brood cells) we were not very successful with worker-laid eggs from our queenright colony. We ascribed our failure to the fact that these WE's failed to remain in the vertical position. They apparently did not possess the surface characteristics of QE's. Therefore, scanning electron micrographs (SEM) were prepared from these eggs, which were compared to those of QE's. Fortunately, we also had available a queenless colony of the same species, where male-producing WE's

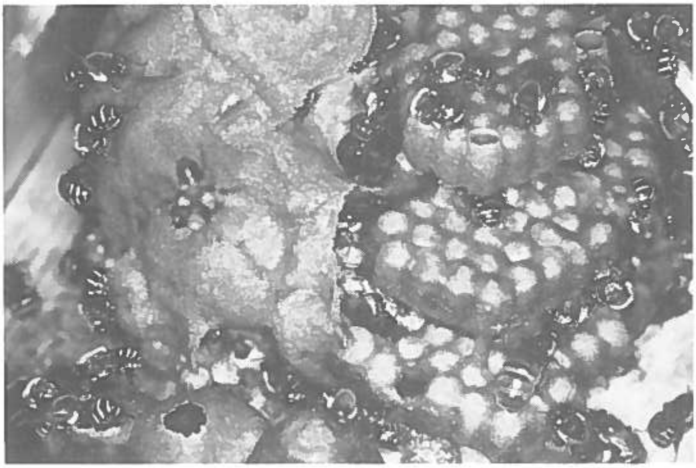


Fig.1a. A typical brood nest of a Melipona stingless bee (Melipona favosa). Three horizontal combs are partly surrounded by a waxen "involucrum" on the left. The upper comb has two ready-built brood cells, protruding with their collar above the comb surface. Three large food storage pots, one closed and two open, are partly visible left bottom. The distinct heteromorphic queen is interacting with a worker on the lowest comb.

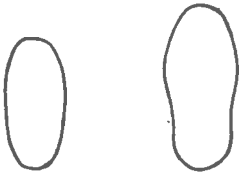
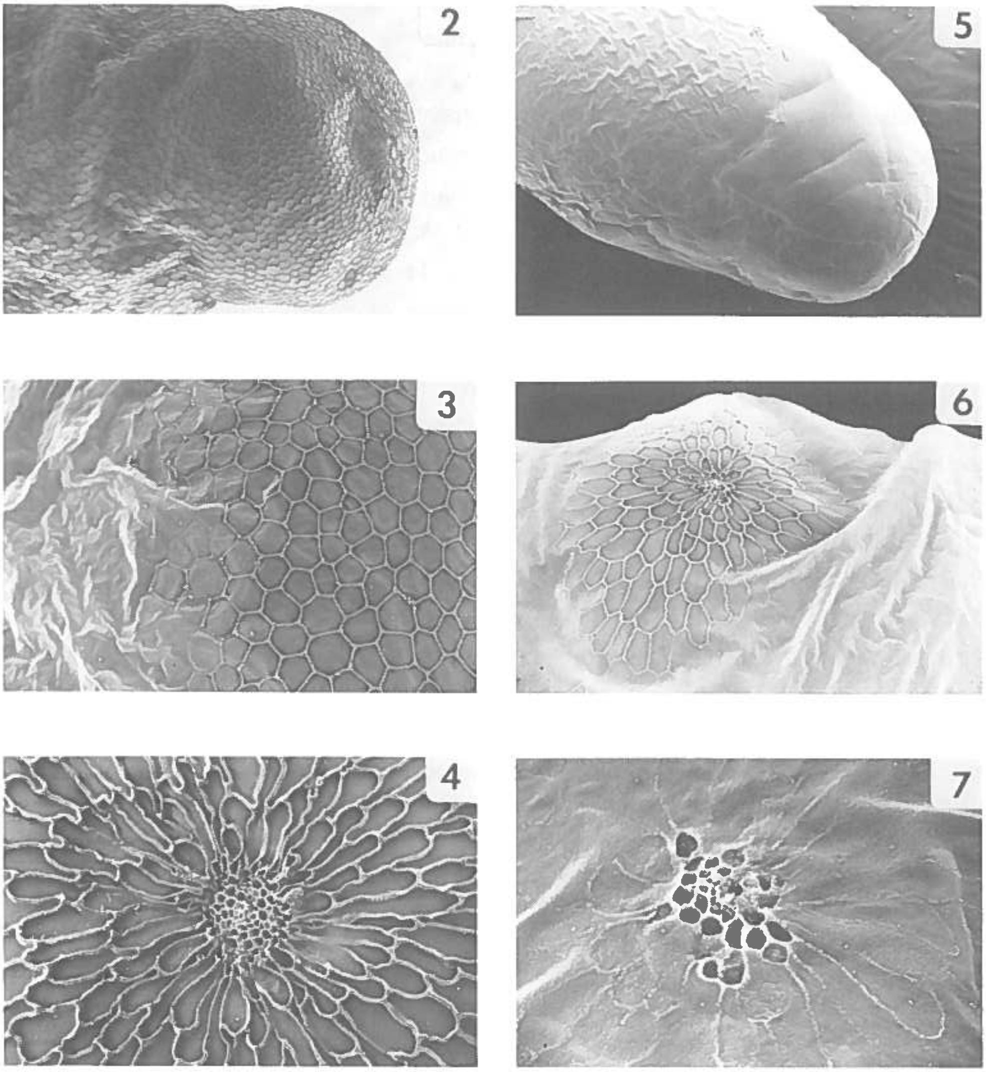


Fig.1b. Typical shape of queen egg (QE), at the right, and smaller queenright worker egg (WE), at the left, of Melipona rufiventris paraensis.

were laid by the orphaned workers. Such queenless WE's could be studied as well.

Although only a small number of worker eggs were collected for SEM, a distinct variation between the different WE's was revealed. The queenless WE's were very similar to the QE's. The length of a QE was 2.8 mm whereas two measured queenless WE's had an average length of 2.5 mm. Both were typically skittle-shaped, and stood erect on the surface of the larval food, with only their flattened base in contact with the liquid (fig.1b). Their vertical position appeared very stable. They wobbled when the comb was shaken but always remained in an upright position. The chorion of these eggs had a very distinct reticulate marking (figs. 2 and 3). The pattern of this network clearly indicates, like a plaster cast, the former position of the follicle cells, which produced this chorion material in the first place. This prominent network is absent from the flattened basal part of the egg (fig. 3). So only the part of the egg that rests on the liquid larval food has a smooth surface. None of the WE's from the queenright colony had this net-like pattern in full. Some of these eggs only had this marking at the top, while on other queenright WE's this pattern extended further downwards, but never as far as observed in the queenless WE's and the QE's. Some queenright WE's even lacked this pattern completely. The smooth part of the queenright WE's varied, but was always present to a considerable degree. In the eggs we examined the relative absence of the pattern was attended with a less distinct skittle shape. These eggs were then ovoid or subspherical, and had an average length of 2.2 mm. These eggs, although they had been placed in the regular vertical position, were very unstable and tended to fall over on the food surface (when we separated the cell from the comb).

The micropyle of both the QE and the queenless WE also appeared to be very similar. This is a structure of about 60 small openings together forming the tip of the egg (fig. 4). This structure is slightly sunken into the surface of the surrounding chorion. A micropyle was not always present on the queenright WE's. Whenever there was considerable network pattern on the chorion of these eggs the micropyle was quite distinct, but smaller than in the QE's: only up to 20 openings were counted. Eggs with a weak or absent chorion network had a similar weak or absent micopyle (figs 6 and 7).



Figs 2-7. SEM photographs of eggs of *Melipona rufiventris paraensis*. 2-4, Queen eggs; 2, net-like chorion pattern and the micropyle at the tip of the egg; 3, transition from marked pattern into smooth bottom part; 4, micropyle. 5-7. Queenright worker eggs; 5, absence of distinct chorion marking; 6, reduced network pattern, only on very top, around the reduced micropyle; 7. much more reduced micropyle and hardly any network pattern on chorion. The rumpled appearance of the egg surface, especially of the smooth parts, results from the fixation and dehydration process.

Discussion

These preliminary results indicate that the WE's laid in the queenless colony, being very similar to QE's and producing males, could be considered as RWE's. The WE's from the queenright colony, showing varying deficiencies in comparison to QE's, could be considered as TWE's. As mentioned above, these eggs are normally eaten by the queen. It is very improbable that these defective TWE's could serve for the production of males, even supposing that they were not all to be eaten by the queen. Their high tendency to topple over on the surface of the larval food makes development very unlikely. However, the degree of the observed morphological differences appeared variable. It is possible that WE's with only minor chorion pattern deviations could have the ability to develop.

We may conclude that the queen of this *Melipona* species exercises control over the reproductive output of her workers by means of influencing the quality of the worker-laid eggs. The precise mechanism of this form of queen-dominance is not yet fully understood. The deficient WE may be a result of partial inhibition or deregulation of oogenesis in queenright workers. It is also possible that the queen stimulates the release of immature worker eggs. This latter type of queen control has also been described for the ant *Myrmica rubra* (Brian & Rigby, 1978). Most likely the phenomenon of a gradual differentiation among WE's also occurs in other congeneric species of *Melipona*.

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VI DISTRIBUTION OF LABOUR AMONG WORKERS OF *M. FAVOSA* F.: AGE-POLYETHISM AND WORKER OVIPOSITION

M.J. Sommeijer

SUMMARY

Division of labour was studied in a colony of *M. favosa* with individually age-marked workers. The average longevity of these workers was 40 days. Various worker-tasks related to reproduction are performed by the same age-group. Construction and provisioning of brood cells, and operculation of provisioned cells, is performed most by bees aged 8 - 12 days. Worker ovipositions occur between 9 - 27 days of age, while workers of 15 - 16 days are most active in this respect. Waste-processing at the rubbish dump is carried out by bees of nearly all ages. Guarding is performed by bees aged 19 - 48 days. From the study of egg-laying behaviour of individual workers, and from the occurrence of ovary development in fixated age-marked bees, we could infer that all workers were once layers. About 50% of the workers may lay one egg and the other 50% may lay two eggs. The laying of three eggs by one worker was observed once. The successive eggs of a single worker may be laid at varying intervals, ranging from 0 - 8 days. Laying workers are not the main provisioners of the cell in which they lay. Regularly they do not even discharge in this cell at all, since they generally lay their eggs at the end of the period of several days in which they are active dischargers.

The evolution of egg-laying by workers is discussed. In this respect reference is also made to our recent finding of an as yet unknown form of queen dominance in bees. This pertains to her control of the quality of the worker-laid eggs. The comparison between the ontogenetic division of labour in stingless bees and honeybees, reveals distinct disparities with respect to the temporal organization of nest activities in individuals and the general occurrence of queenright worker oviposition.

INTRODUCTION

Many recent behavioral studies have focused on the differentiation of behaviour within social groups. In insects this phenomenon is considered fundamental to the evolution of polymorphism within colonies of eusocial forms (MICHENER, 1974; WILSON, 1975). From the early publication of "The Feminine Monarchie" by Charles BUTLER in 1609, until very recently many articles on the division of labour in *Apis mellifera* colonies have appeared. During their life-time honeybee workers of normal in-season colonies perform a characteristic sequence of activities according to their age. These different age-tasks are related to the temporary activation of different glandular systems in the workers. This division of labour according to age has, however, a considerable plasticity (SEKUGUCHI & SAKAGAMI, 1966).

Polyethism, the behavioral specialization among members of a society, has also been studied in ants (cf. OSTER & WILSON, 1978; BRIAN, 1979; WILSON, 1971, 1976, 1980). In many ants with their morphologically different subcastes combined with age-based division of labour polyethism is more elaborate.

Our knowledge about behavioural specializations within colonies of stingless bees (Apidae; Meliponinae) is still meagre, and the need for data concerning this eusocial group has been stressed recently (SEELEY, 1982). The purpose of this paper is to present further data on this subject. The first study on division of labour in stingless bees was by BASSINDALE (1955), who recorded the activities of differently melanized workers in *Trigona braunsi (=gribodoi)*. There are later studies by KERR & SANTOS NETO (1956); HEBLING, KERR & KERR (1964), DARCHEN (1970), and BEGO & SIMOES (1972, 1979). Some of these studies are characterized by the acceptance of a rigid ontogenetic sequence of labour tasks in *Apis* workers. In the behaviour of the studied stingless bee workers only minor differences are distinguished in this respect. In our previous study of the distribution of labour in individual workers of *Melipona favosa* we observed a fundamental difference in the temporal organization of their behaviour compared to that of the honeybee. We established that in a single worker larval feeding and brood cell construction are temporally tightly linked activities (SOMMEIJER, BEUVENS & VERBEEK, 1982). For that paper we were able to study the activity of individuals in colonies in which all the bees were marked individually in one single operation on the same day. The present paper deals with our observations on the division of labour in a colony from which we continuously marked all new bees individually as they emerged. Consequently we were able to record the individual participation of workers of various ages in the respective nest-activities and foraging. At the termination of the observations we compared the ovarial development of all workers living at that moment.

MATERIAL AND METHODS

A colony of *Melipona favosa*, collected on February 26th, 1979, at Balandra, Trinidad, West Indies, was transferred to an observation hive for intranidal observation in an annex of the Commonwealth Institute of Biological Control at Curepe, Trinidad. The colony comprised about 275 bees. The bees had an exit from the hive via a 30 cm long plastic tube leading through the wall of the room. Outside, the opening of this tube protruded 2 cm from the wall, and for better orientation of returning bees, it was marked with a blue circular disk. So that the individuals participating in the various activities could be recorded as they grew older, all bees were marked within 24 hours of their emergence. Thus, after a few months, we had obtained a colony consisting entirely of individually age-marked workers. The first bees were marked for this observation series on May 22nd. By July 27th, the last observation day, a total of 507 individuals had been marked; bees were found to have emerged at a rate of 7.7 bees (SD=2.0) per day. On that date the colony comprised 274 bees.

A number of distinctive worker activities were selected for recording. These behaviours were registered through direct observation under red light. The following worker activities were recorded:

- building brood cells: mainly mandibular "manipulation" of cell parts or working inside the cell
- provisioning cells with larval food during the "provisioning and oviposition process (POP)"
- ovipositing on top of the larval food after the cell had been filled with this liquid (generally this egg is eaten by the queen after being laid, which is then followed by the queen's oviposition)
- sealing the cell after oviposition by the queen
- working at the waste-dump; "manipulating" particles of dead bees, cell re-

mains etc. with the mouth parts
- guarding; the only behaviour recorded outside. Guarding bees are positioned in the exit opening
- foraging; all bees returning to the hive, either with pollen or nectar, were registered.

The observations were carried out every other day, in principle for 12 hours daily, from 05.45 to 17.45. There were 28 observation days from June 4 to July 27. Every hour the activities of individuals participating in the selected behaviours were recorded as follows: 15 min. observation of cell building bees; 15 min. observation of waste-manipulating bees; 15 min. observation of returning foragers; from the outside one point recording was made of the guarding individuals in the exit hole; the observer was allowed a few minutes rest. During each of the 15 min. observation blocks, building and waste-manipulating individuals were listed and therefore only scored once. The recording of POP activities had priority whenever POP sequences took place. On these occasions we registered the individuals occupied in discharging larval food, oviposition and operculation. Besides the every-other-day recording of individual bees performing the selected behaviours, we also recorded which workers participated in POP on some of the remaining days. These additional recordings of POP sequences on intervening days were made during observation sessions for other behavioural phenomena, and were consequently less systematic and complete. The ovarian conditions of the bees were studied after the fixation of all individuals in Bouin's fluid at the end of the observation period. Total preparations of the ovaries, embedded in Berlese's mixture, were analyzed microscopically. The width of the ovaries was measured. The stages of ovary-development were observed and the occurrence of well-developed ovaries, which contained distinct ova, was analyzed in relation to the age of the animals.

RESULTS

Age-based division of labour

The total number of scores collected were quite different for the various behaviours. This can be ascribed to the different frequencies of these behaviours and the different methods of recording them. The most scores were

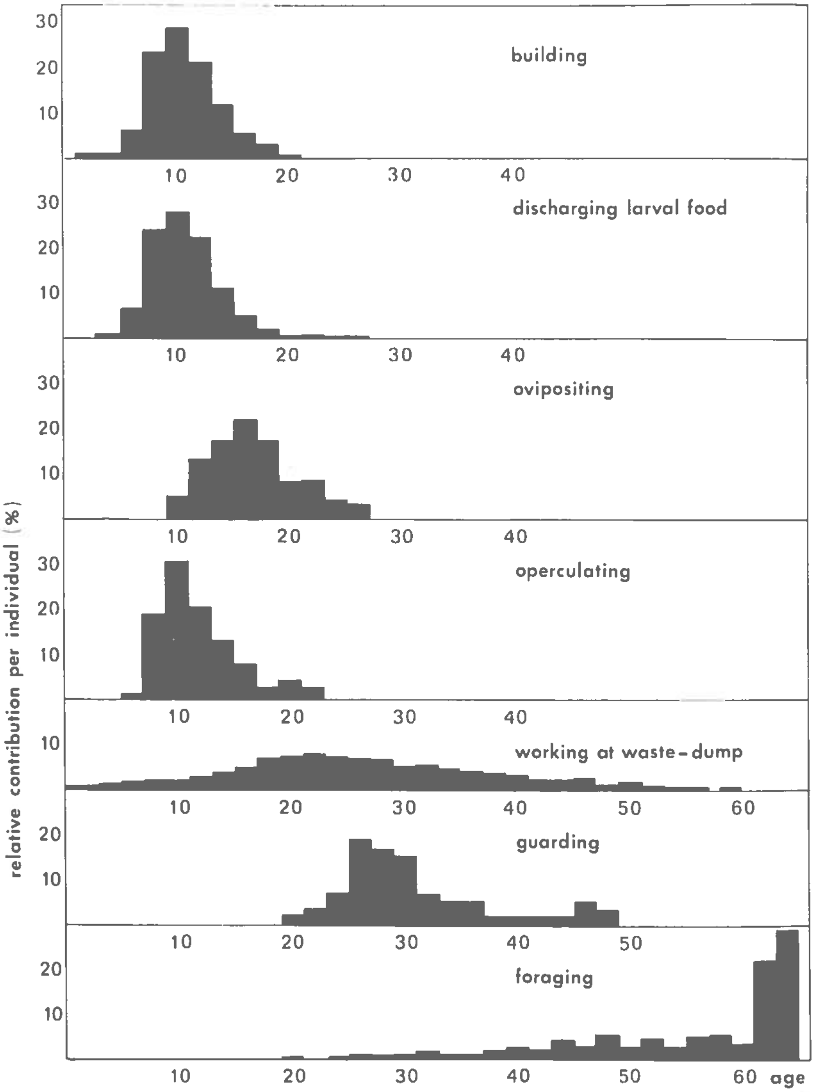


Fig. 1. Age-dependent division of labour in workers of *Melipona favosa*. The relative individual contribution per bee of each successive age-category is indicated. There was only one day of difference between the ages of bees in each age-category.

taken for waste-manipulating and for constructing bees, 6578 and 4460 respectively. For discharging larval food the figure was: 2085 scores; for ovipositing: 117; for cell operculating: 144; for guarding: 131; for coming in with nectar or pollen: 205. The behavioural performance of workers of different age was measured by analyzing the average individual contribution for different age categories. There was only one day of difference between the ages of bees in each category. The scores for the recorded behaviours were corrected for the number of bees present in each age class. The relative individual contribution per bee of each successive age group is shown in Fig. 1: this reveals that in reproductive nest activities animals from a rather narrow range of age categories take part. For the different nest activities these are almost the same age categories; three activities, namely building brood cells, depositing larval food, and operculating are performed most at the same modal age of 9-10 days. It is at that age that the first bees start to oviposit. The maximum activity for egg-laying appears, however, a little later on 15-16 days, while the oldest laying workers are 26-27 days. The bees that work at the waste-dump belong to nearly all age categories. This activity was performed most by 21-22 days old bees, but there was no distinct peak for this. Guarding was performed by bees from 19-48 days, without a very definite peak within these limits. Guarding bees, who are situated in the nest entrance, persist in this position for a considerable time. They let departing bees and arriving bees pass by, and react aggressively to other disturbances, e.g. they bite on a blade of grass poked inside. Foraging behaviour, measured by recording the bees entering with pollen or nectar, starts at about the same age. However, at this age the relative participation of bees is still very low; it increases slowly to reach a peak in bees aged 62 and over.

Longevity of the workers

The average longevity was calculated for a group of workers that were introduced long enough before the final observation to have all died before that date. For this calculation it was assumed that a bee's final day of life was the last day on which it was observed in the colony. The bees used for this measurement had been introduced between the beginning of observations, i.e. 77 days before the last day of observation, and the 57th day before the last day. The workers of this group of 21 bees had an average longevity of 40

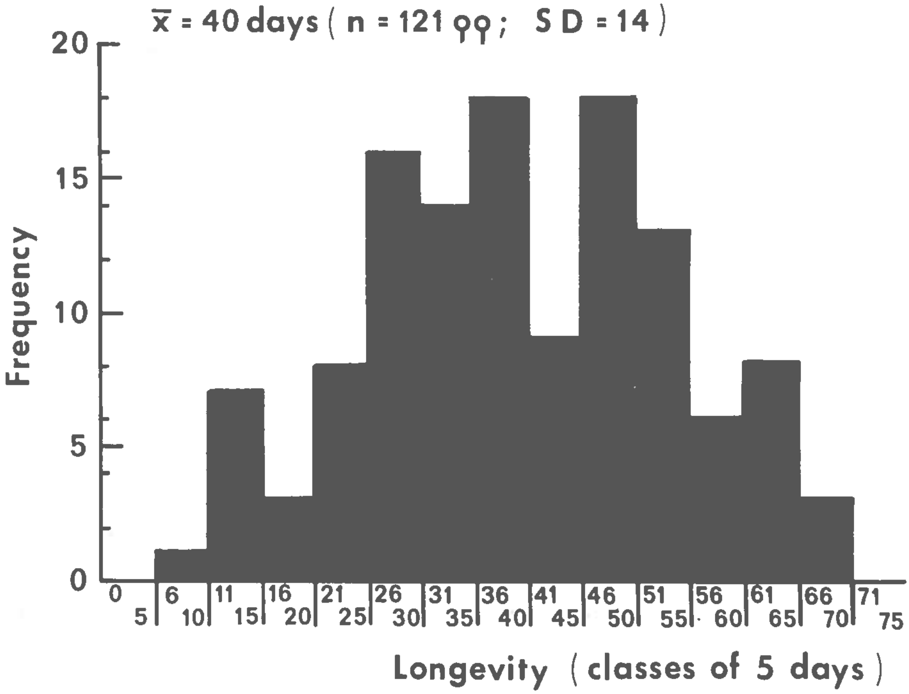


Fig. 2. The longevity of workers of *Melipona favosa*. The average longevity was calculated for the group of 121 bees which were introduced first. These bees had all (except one) disappeared from the colony before all bees were fixated at the end of the observation period. We assumed that the last day on which a bee was observed in the colony was her final day of life.

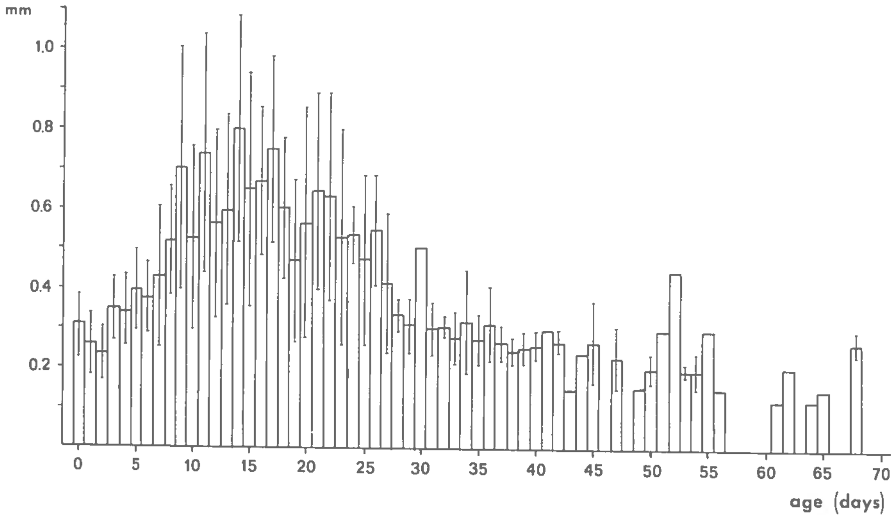
days. The oldest bee included in this group was the only one of these who was still alive at the termination of the observations. By then this bee had reached an age of 68 days (Fig. 2).

Individual egg-laying behaviour of workers

As shown above, egg-laying behaviour is performed by workers aged from 9 to 27 days. The analysis of the distribution of ovarial development among all the bees alive at the termination of the observation revealed that full ovary development is also restricted to this period of age. Within these age-limits ovary development is very common (Fig. 3). It was further evident from the number of eggs laid by individual bees that most of these bees, if not all, participate in the laying of eggs. Of the 102 recorded laying workers 88 (86%) laid only once. Two ovipositions were recorded for 13 workers (13%), and only one worker (1%) was seen to lay three eggs. In order to obtain further quantitative information on the individual oviposition of workers, we analysed the scores for egg-laying of all workers of 25 days and older, which were still alive at the end of the observations. Of these 86 bees, whose ages ranged from 25 to 68 days, we had observed 28 to be laying workers; only 3 of these workers laid twice. These scores were obtained for observations covering only 25% of the total time. Thus, if all bees in general lay only one egg, we would expect to see 22 workers laying once; if they all had laid twice then we would have observed a total of 39 laying workers and five of these would have been seen to lay two eggs. The observed rate of egg-laying appears to correspond to the assumption that 50% of the bees lay only one egg, and the other 50% lay two eggs. On the basis of chi-square comparisons, we could rule out the possibility of more discrete differences in individual rates of egg-laying among the workers.

The workers which were observed ovipositing more than once laid these successive eggs at varying intervals, ranging from 0-8 days. The periods between the first observed oviposition and the day these workers were fixated, while still having fully developed ovaries, ranged from 2-11 days. The only bee that was observed laying three eggs laid her first observed egg when she was 11 days and the last two on the same day, when 18 days old (Table 1).

fig. 3


A. average ovary width

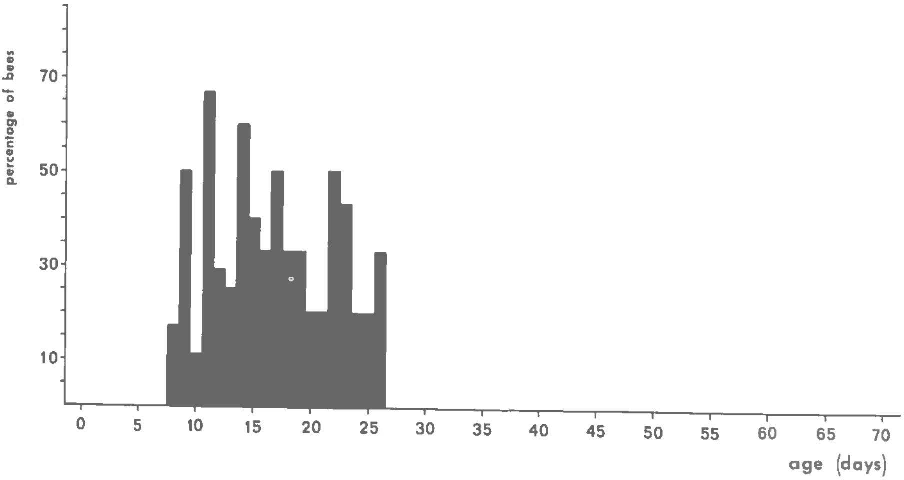

B. occurrence of well-developed ovaries

Fig. 3. Ovary-analysis of the whole fixated colony. A. Average ovary-width in all the workers of the various ages. B. Occurrence of well-developed ovaries (with distinct ova) in percentages of the bees of the various ages.

age at:		
first oviposition:	second oviposition/ fully devel. ovaries (*):	third oviposition:
10	14	--
11	11	--
11	13	--
11	18	18
11	18	--
12	17	--
12	20	--
13	15	--
13	21	--
14	21	--
14	21	--
16	20	--
16	22	--
17	18	--
9	11*	--
9	21*	--
10	15*	--
13	23*	--

Table 1. Age-dependent oviposition behaviour of single workers: ages at which bees performed their first, second (and third) observed ovipositions.

Percentages of workers that laid in a POP who had also been observed as dischargers in previous POP's, in the same POP, or in later POP's.

Discharged in POP:	% of layers
previous and same	40
exclusively previous	40
same and later	--
exclusively later	--
previous, same and later	10
exclusively same	9
previous and later	1

Table 2. Sequence of individual egg-laying and discharging activity in successively observed POP's.

The discharge of larval food by laying workers in successive POP's

As we have seen above, there is a discrepancy in the ages at which eggs are released and at which the other recorded POP activities are performed most. To find out whether this is also true for individual workers, we analyzed the sequential occurrence of egg-laying and discharging larval food in those individuals (n=91) who were observed to perform both behaviours during the total observation period. From table 2 it is evident that workers did indeed tend to lay their eggs in later POP's than those in which they discharged brood food frequently.

The discharge of larval food by egg-layers within a single POP

Elsewhere we have reported that only 58% of all POP's contained one or more worker ovipositions (SOMMEIJER, BEUVENS & VERBEEK, 1982). We have seen now that laying workers release their eggs at the end of their discharging period and often also in POP's other than those in which they discharge. However, it appeared that in 72% of all POP's containing a worker oviposition, the egg was laid by a discharger in this POP. This raises the question of whether egg-layers are characterized by a specific discharge performance or other POP behaviour, in POP's in which they carry out both activities: provisioning and oviposition. Elsewhere we have reported that laying workers in queenless colonies initiate the discharging sequence by carrying out the first discharge (SOMMEIJER & VELTHUIS, 1977). The present analysis revealed that this is different in queenright POP's. Bees that discharged and oviposited in the same POP did not participate intensively in the provisioning of that cell (for classification of discharging activity, cf. SOMMEIJER, BEUVENS & VERBEEK, 1982). In this series of 67 POP's in which the egg-layer also discharged, she was never among the first two dischargers. (In other observations this was, however, sporadically the case.) Such layers usually discharged at the end of the sequence of on the average 12 discharges, in which about eight bees took part. This egg was then generally laid immediately after their own first and only food regurgitation in this POP.

The worker ovipositions did not always terminate the provisioning sequence. Regularly, one or more regurgitations by other workers followed after the queen had devoured the laying worker's egg. Only in 44% of these POP's with worker oviposition was the sequence of discharges concluded with the laying of a worker egg. Of these final eggs 51% were released by a discharger in the same POP.

DISCUSSION

From these results we can conclude that the division of labour in the worker class of *M. favosa* is in principle age-dependent. This is in agreement with the finding of the authors listed in the introduction. The two activities of building and provisioning of brood cells are both performed most by the same age-category; this is in agreement with our previous finding of the strong correlation of these behaviours in individual bees. The fact that these activities are carried out by bees of the same age-categories has also been found for *Melipona quadrifasciata* (KERR & SANTOS, 1956), *Apotrigona nebulata* (DARCHEN, 1969), *Melipona rufiventris* (CECCATO, 1970), and *Scaptotrigona postica* (BEGO & SIMOES, 1972). Besides, bees aged 9 and 10 days most frequently operculated "serviced" brood cells. It is evident from these data that workers lay their eggs generally at a later age than the age at which the above-mentioned activities are carried out frequently. However, contemporaneous egg-laying does occur. Besides, ovary development is considerable among bees of age-categories where discharging per bee is at its most active.

From the analysis at individual level we can conclude that the most active provisioners of a particular cell, who are also the most important constructors of this cell (SOMMEIJER, BEUVENS & VERBEEK, 1982), do not generally oviposit in this cell. Worker eggs are generally laid by slightly older bees who regularly performed their major discharging activity in previous POP's. Nevertheless these older bees are often among the minor and last provisioners of the cell in which they lay. It is remarkable that worker eggs are laid at an age which is well before the age at which foraging starts. This fact, together with the note-worthy combination of cell construction and cell provisioning that goes on when the bees are a few days younger, indicates the secondary specialization of the mass-provisioning of stingless bees. In this connection it is of interest that stingless bees mass-provision their cells with liquid food whereas solitary and semi-social bees mass-provision their cells with solid pollen masses (VELTHUIS, pers. comm.). The simultaneous activation of the glandular systems for brood production (BEGO & SIMOES, 1972), however, resembles the condition of solitary bees, and is strikingly different from the successive activation in honey-bees.

The phenomenon of laying workers in queenright colonies of stingless bees deserves a great deal of attention. Through the haplo-diploid sex determination system, unmated workers may produce offspring by the laying of unfertilized eggs. Such eggs can only produce males. It is known from many species of stingless bees that worker eggs are eaten by the queen. MICHENER (1974) states: "probably the most interesting part of the cell-provisioning and egg-laying process in stingless bees is the laying of trophic eggs". It has been found that workers of certain species of the Trigonini produce special sterile trophic eggs, in addition to fertile male-producing eggs (e.g. BEIG, 1972; reviewed by SAKAGAMI, 1982). From our recordings of egg-laying behaviour during a quarter of the total time, and the general occurrence of the highest stage of ovary activation in fixated workers of a specific age-group, we conclude that all workers of *M. favosa* are potential layers. It is important for the relation between queen and workers, which is a competitive one, that these temporary layers are in part the same bees as the constructors and provisioners of brood cells. In most species of stingless bees, the queen controls worker reproduction by the elimination of worker-laid eggs through oophagy. In many species this queen-worker interaction occurs in varying forms of egg-eating behaviour. I agree with KERR (1969) that this oophagy perhaps evolved into the particularly complicated type of queen-feeding behaviour seen in some groups. The laying of enormous alimentary sterile worker eggs of *T. (Scaptotrigona) postica* is an example of this.

A remarkable form of control by the queen is indicated by our recent finding of morphologically different worker-eggs in *Melipona*. We found that in *M. rufiventris paraensis* worker eggs differ according to the conditions under which they are laid (SOMMEIJER, DOHMEN & VAN ZEIJL, in press). The eggs laid by queenright workers were different from and more fragile than the male-producing eggs laid by queenless workers. However, the extent to which the queenright worker eggs differed from normal eggs varied. They ranged from very aberrant, to nearly the same as queen eggs. Perhaps viable reproductive worker eggs are sometimes laid, even in a queenright situation, possibly at a later stage, or under specific colony conditions.

The plasticity of the age-based labour distribution, which is known for the honeybee, has also been reported for stingless bees (BEGO & SIMOES, 1972). We also saw nice examples of this during our first observations in individually marked colonies. As a consequence of inadequate manipulation these colonies were in poor condition and lacked the continuous supply of emerging bees. In these colonies we observed that workers which had participated in cell provisioning for some days, re-appeared as dischargers a few later. At the time this observation made us even think that there existed a specific group of workers permanently designated for this task. Apparently, the period during which bees may participate in a certain, in principle age-dependent activity, also depends on the conditions of the colony, such as the rate at which young bees emerge.

Whereas certain activities were restricted to narrow age-categories, other behaviours, especially the processing of waste, could be performed by most age classes. The same has been reported also by other authors (CECCATO, 1970). However, in our data this could perhaps also be a result of not having sufficiently discriminated various occupations within this activity.

Our finding that foraging is the major activity for the older bees is in full agreement with other studies (see INTRODUCTION). The same has been reported for honeybees. In our previous study on the foraging behaviour of various stingless bees, we found that workers of *M. favosa* do not demonstrate a specific ontogeny for the collection of the pollen and nectar (SOMMEIJER, ROOY, PUNT & DE BRUIJN, in press).

The observed average longevity of 40 days may be an under estimate, since the mortality observed in the younger bees of the sample may have been increased by our handling for marking etc.. Nevertheless, the longevity of these workers was longer than is known for in-season workers of the honeybee. This is in agreement with results obtained for another stingless bee *Plebeia droryana* (TERADA, GAROFALO & SAKAGAMI, 1975). In other colonies of *M. favosa* we commonly observed bees that were 60-70 days old; the juvenile stages of these bees were more extended than those of the honeybee (SOMMEIJER, unpubl.). In view of the relatively long lives of the few studied stingless bee species it should be realized that the presence of elderly workers is not necessarily an indication of a good, or even normal colony condition. It is a virtual truism that honeybee workers are most short-lived in strong, thriving colonies, this may be true for stingless bees as well. In some of our laboratory colonies with weak populations of *M. favosa* we observed workers aged up to 120 days.

This study reveals that older stingless bee workers are the foragers and have little or nothing to do with brood care. This is similar to division of the labour in honeybees, however, this age-based task assignment applies to nearly all social insects. The division of labour in stingless bees, however, differs essentially from that in honeybees as far as the production and care of brood are concerned, since stingless bees and honeybees have quite different mechanisms for feeding the larvae and the queen. The general occurrence of frequent worker ovipositions in stingless bees further accounts for the distinctive form of polyethism in these bees.

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VII THE SOCIAL FOOD FLOW WITHIN THE COLONY OF THE STINGLESS BEE, *MELIPONA FAVOSA* (F)

M.J. Sommeijer, L.L.M. de Bruijn and C. van de Guchte

SUMMARY

Trophallaxis in workers of stingless bees was studied in colonies of *Melipona favosa* with marked individuals. In the brood area of the nest all trophallactic interactions are initiated by the soliciting bee, whereas spontaneous offerings occur particularly near the nest exit. The trophallactic network within the colony does not represent an open system that leads to even distribution. The cell-provisioning bees obtain food, mainly from other bees. Since cell-provisioning is strongly age-dependent, the trophallactic benefit changes strongly according to the age of the bees. The bees that discharge on a particular day are not involved in the pollen uptake from food pots on that day. The dischargers probably obtain trophallactically a liquid food suspension with a high pollen content, which they subsequently regurgitate into the brood cells. The dischargers also perform active food solicitations with other bees immediately after they have discharged during the progress of the provisioning of a cell. For this they often depart quickly from the cell that is being filled. We assume that their withdrawal from the cell vicinity enhances their chances to meet quickly bees that are less reluctant than the other dischargers nearby to donate food to them. This behavior sometimes enables dischargers to perform subsequent regurgitations in the same cell.

INTRODUCTION

Trophallaxis, the exchange of alimentary liquid among members of a colony of social insects, can either be mutual or unilateral. This exchange of liquid food occurs in two patterns: the passing on of larval saliva to attending adults and the exchange of liquid between adults. After extensive observations of this behavior in several ants, the honeybees and the termites many investigators took the view that the social food flow in colonies of eusocial insects represents an open system (WHEELER, 1928; LE MASNE, 1953; WILSON, 1971; COLE, 1981). Especially when the colony was regarded as a superorganism it was convenient to consider the complete network of trophallactically interacting individuals as a "social stomach system". One of the main functions of such a system is indicated in the important review by WILSON (1971). He states: "the lack of selfishness on the part of the individual honeybee and ant workers and the ease with which individuals shift from one role to another ensures a rapid and relatively even distribution of liquid food through the colony". However, in certain ants the trophallactic system is not so open, since selective regurgitations to specific members of the colony are observed (WILSON, 1974). A very uneven distribution of liquid food has recently been described by COLE (1981) for colonies of the ant *Leptothorax*. This author noted the occurrence of distinct unidirectional food transfers towards high-ranking workers in the reproductive dominance hierarchy of the queenright workers. It has long been known that the trophallactic system in social wasps differs greatly from an open system (PARDI, 1948; MONTAGNER, 1966). Among these wasps the direction of the social food flow depends largely on the dominance hierarchy of the females in the nest.

MICHENER (1974) considered food exchange among workers of highly eusocial bees as being at the heart of the social systems of these insects. Extensive exchange of liquid food in honeybee colonies has been demonstrated by studies involving the use of radioactive syrup (NIXON & RIBBANDS, 1952). The apparent quick spread of this syrup over all individuals of the colony was suggestive of the integrative function of the trophallactic system, which allows, for example, for the continuous dispersion of queen pheromones. BUTLER (1974) stressed, however, that food transmissions in honeybee colonies do not occur at random. Recently, KORST & VELTHUIS (1982) found that the organization of the trophallactic network in small groups of *Apis mellifera* is correlated with the differential ovarial development in the members of these groups.

Trophallactic behavior in colonies of stingless bees is still poorly documented, although the need for such documentation in order to study social behavior was already recognized by KERR in 1969. Some observations on trophallaxis in these bees were made by SAKAGAMI, ZUCCHI and co-workers (cf. SAKAGAMI, ZUCCHI & PORTUGAL-ARAUJO, 1977), but these remarks mainly concerned queen-worker co-actions. They found that food-begging by the queen is pronounced in most stingless bees, although definite deliveries to her are rare.

We have presented quantitative data about this peculiar trophallactic behavior for the queen of *M. favosa* (SOMMEIJER & DE BRUIJN, in press; SOMMEIJER, 1981; SOMMEIJER, in press). There are no quantitative data available about regurgitations between workers, although trophallactic transmission of food to other workers by returning foragers (LINDAUER & KERR, 1958) and by workers who collected syrup, nectar or honey has been observed (SAKAGAMI & ONIKI, 1963).

In this paper we present quantitative data on the patterns of the mutual trophallactic activity of workers of the stingless bee *Melipona favosa*. Stimulated by our earlier observation that the same worker carries out more than one food regurgitation during the provisioning of the same brood cell (SOMMEIJER, 1979; SOMMEIJER, BEUVENS & VERBEEK, 1982), we became interested in the trophallactic behavior of these workers during the mass-provisioning period. During subsequent observations we also studied the trophallaxis activity in other periods. The latter observations were carried out in colonies with age-marked bees.

Pollen is a major component of the liquid larval food that is regurgitated by a limited number of bees during the mass-provisioning of the brood cell. The participation by individuals in the ingestion of pollen taken from the storage pots was studied in order to obtain information about the influx of this material into the trophallactic system and its subsequent release as larval food.

MATERIAL AND METHODS

Colonies of *M. favosa* were housed in observation hives and the bees were individually marked at emergence. The trophallaxis of individuals was recorded during the "extra-oviposition period" and during the "oviposition period" (cf. SAKAGAMI, ZUCCHI & PORTUGAL-ARAUJO, 1977). We applied the following definitions:

POP - "provisioning and oviposition process"; short behavioral sequence of cell provisioning and egg-laying
TC - "trophallactic coaction" between two bees; this can either be a "transfer" (with a proper transmission) or an "unrewarded solicitation"
TA - "trophallactic act" of a single bee
TI - "trophallaxis index"; trophic benefit is indicated by value per age for rewarded solicitations minus donations (also negative values), divided

by the sum of these

DM - "discharging-moment"; indicates for each TA of single bees the relation to the moment of cell provisioning in days. A positive or negative DM indicates the number of days on which the nearest (observed) larval food discharge of this bee had taken place respectively after of before the actual TA.

PF - "pollen-feeding"; the uptake of pollen from storage pots

IDI - "inter-discharge interval"; for bees who discharge more than once in a single POP, the period between their first and last discharge.

Three series of observations were made. One age-marked colony from Surinam was continuously observed in Utrecht to register all "provisioning and oviposition processes (POP's)" that took place during a period of 13 successive days. This hive had no excit to the outside. Here we recorded, as far as possible, the transfers with a minimum duration of about two seconds between workers, and between workers and males in the total brood compartment. The recordings of these were made in 45' episodes of direct observation, one every three hours, during a daily "intensive observation period" of 9-10 hrs. A total of 253 transfers could thus be registered, 196 of which were between two individuals that were properly marked. Additionally, the bees that were feeding on pollen in the food stores were registered here four times, namely every 15 min. during each of these observation episodes.

Another observation series was carried out in an age-marked colony in Trinidad (West-Indies). This colony had a free exit. Here TC's were recorded during 13 non-successive observation days. On four of these days we recorded exclusively and continuously trophallaxis activity throughout daylight hours. On the other days we registered TC's during shorter periods (each not less than 30 minutes) between other observations. In these observations we attempted to register all occurring TC's, either rewarded or not. During the total observation time of 41 hours 48 minutes a total of 2122 trophallactic interactions were scored here. We related the trophallactic activity of the individual bees to their age, and also to the nearest moment, in future or in the past, when they were observed to participate in discharges of larval food in brood cells. In this series the behavior of bees consuming pollen from the food stores was recorded during only one day. All bees that were feeding on pollen in open pots were recorded from 9.00 - 17.00 hrs. On the same day a second observer recorded the behavior of the discharging bees during the three POP's that occurred in this period.

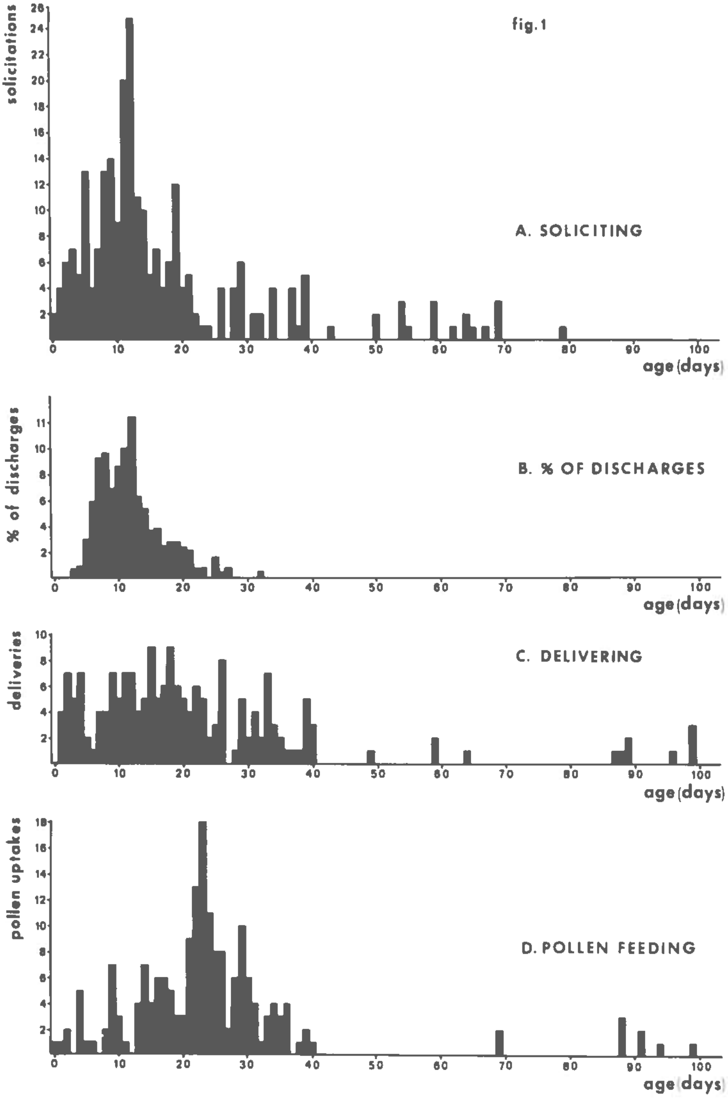


Fig. 1. Age dependence of trophalaxis and related behaviors. A. rewarded solicitations; B. larval food discharges in brood cells (in percentages); C. delivering after being solicited; D. pollen feeding from storage pot.

In the third series of observations, carried out in Utrecht, we specifically studied trophallaxis during the provisioning of brood cells. This was done by video recordings of the behavioral sequences of the POP. With this method only a restricted area of the comb could by surveyed, the main focus being on the brood cell. These observations were directed to the TA's of workers that were involved in the discharging of larval food into the cell. Soliciting and donor behavior was related to the larval food discharging performance of these bees.

RESULTS

Trophallaxis behavior of non-foraging bees always involves two individuals. The soliciting bee lifts its head distinctly. With the glossa, stretched out from between the slightly open mandibles, and often also the forelegs, she actively touches the face of the approached nest mate. Bees that are solicited in this way may donate food by lifting the head a little, opening their mandibles wide and regurgitating food. The actual liquid is generally difficult to observe. A quite different response occurs when the solicitation is not "rewarded". The solicited bee then crouches with a distinct ventral turning of the head.

The observations revealed that house-bees do not offer food to nestmates spontaneously. Spontaneous offerings are performed exclusively by returning nectar foragers. Thus, all trophallactic co-actions between non-foragers are always initiated by the soliciting behavior of one of the participants.

For the first observation series we analysed the age distribution for the soliciting bees. This revealed that successful solicitations were most actively performed by the bees that were of the same age as the most active dischargers of larval food in brood cells (Fig. 1). For both activities the maximum frequency was reached by bees aged between 8 and 15 days. The age distribution for the trophallactically donating bees did not show such a distinct optimum. Bees aged between 3 and 28 days participated in delivering to more or less the same degree. The uptake of pollen was performed most by bees aged between 22 - 30 days (Fig. 1).

The non-randomness of trophallaxis induced us to study the relation between cell provisioning and trophallactic advantage. For this purpose the bees which performed the recorded TC's were placed in a number of categories.

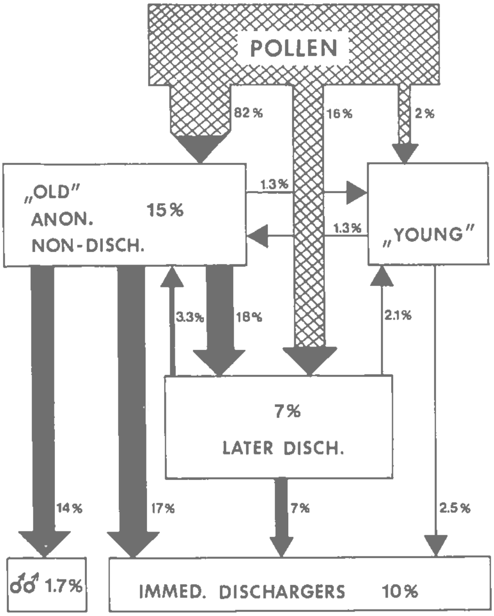


Fig. 2. The social food-flow in a colony of *M. favosa*. The direction of the food-flow is indicated by the arrows. Their width indicates the number of transfers from bees of one category to bees of another category in percentages of the total number of 253 transfers*. The following categories are taken separately:

A. "immed. dischargers", discharged in the POP which immediately preceded or immediately succeeded the recorded transfer
B. "later disch.", bees observed to discharge later, but who were at moment of transfer older than youngest observed dischargers (4 days, in this series)
C. "young", bees who were younger than youngest observed dischargers
D. "old, anon., non-disch.", bees older than oldest dischargers, together with unidentified bees, and bees who no longer participated in discharging, but who were between the ages of youngest and oldest observed dischargers
E. "♂♂", males

The participation of bees of these categories in the uptake of pollen from the storage pots is indicated by hatched arrows, according to the same convention (n= 186 visits to the pollen pots).

*Food-flows of less than 1% are omitted for reason of clarity. The percentages shown within the group-blocks represent the number of transfers between individuals of the same groups.

The following categories were formed: A. bees that discharged in the cell immediately prior to or following the recorded transfer (the "immediate dischargers"); B. bees that were observed to discharge later, but who were at the moment of transfer older than the youngest observed dischargers (four days, in this series); C. bees that were younger than the youngest observed dischargers; D. bees that were older than the oldest observed dischargers, together with the unidentified bees, and the bees which no longer participated in discharging, but which were between the ages of youngest and oldest observed dischargers; E. the males. The trophallactic food-flow between these groups is shown in Fig. 2; the figure shows the direction of food transmission for the 253 transfers.

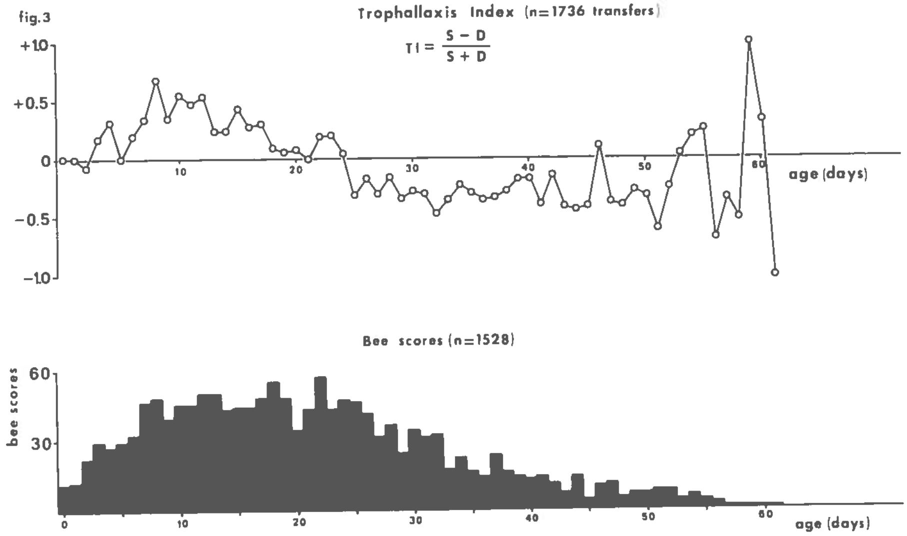
It is evident from this figure that there was a unidirectional flow of food from the pollen supply towards the "immediate dischargers" and the males, via the three remaining categories. All categories except the males played a part in delivering food towards the "immediate dischargers". "Immediately discharging" workers did not take pollen directly from the pollen pots.

From the observations in Trinidad we obtained fewer recordings of the unrewarded solicitations (386) than of transfers (1736). This does not necessarily indicate that the unrewarded solicitations occurred less frequently. The unrewarded TC's last for a much shorter time (fractions of seconds) than the rewarded ones (several seconds). Therefore, the unrewarded TC's may simply be missed more often by the observer.

We did not know whether the bees participating in the unrewarded solicitations are different from the ones participating in transfers, in that they possibly did not ever discharge larval food. It appeared that they were not. In 38% of all transfers and in 34% of all unrewarded solicitations, both individuals of the TC were observed to discharge in a cell during the observation series.

Fig. 3 shows that the trophallactic benefit changes with the age of the bees. The trophallaxis index shown in Fig. 3 indicates the value per age for solicitations minus donations, divided by the sum of these. The analysis of age-dependence of trophallactic exchange was extended to the age-relations of partners in a TC. This revealed (Fig. 4) that successful solicitations are till about an age of 30 - 40 days principally (for more than 50%) directed towards bees older than the solicitor.

Deliveries are already from the age of 8 days mainly (for more than 50%) made to younger bees. This difference in age between the soliciting and



<u>Fig. 3</u>. A. The trophallaxis index for food transmissions indicates for each age the value for occurring solicitations minus donations, divided by the sum of these. B. Frequency distribution for scores of bees engaged in food transmissions per age.

fig.4 **Trophallaxis in Melipona favosa**

Age dependent donor and successful soliciting behavior (n=1736 transfers)

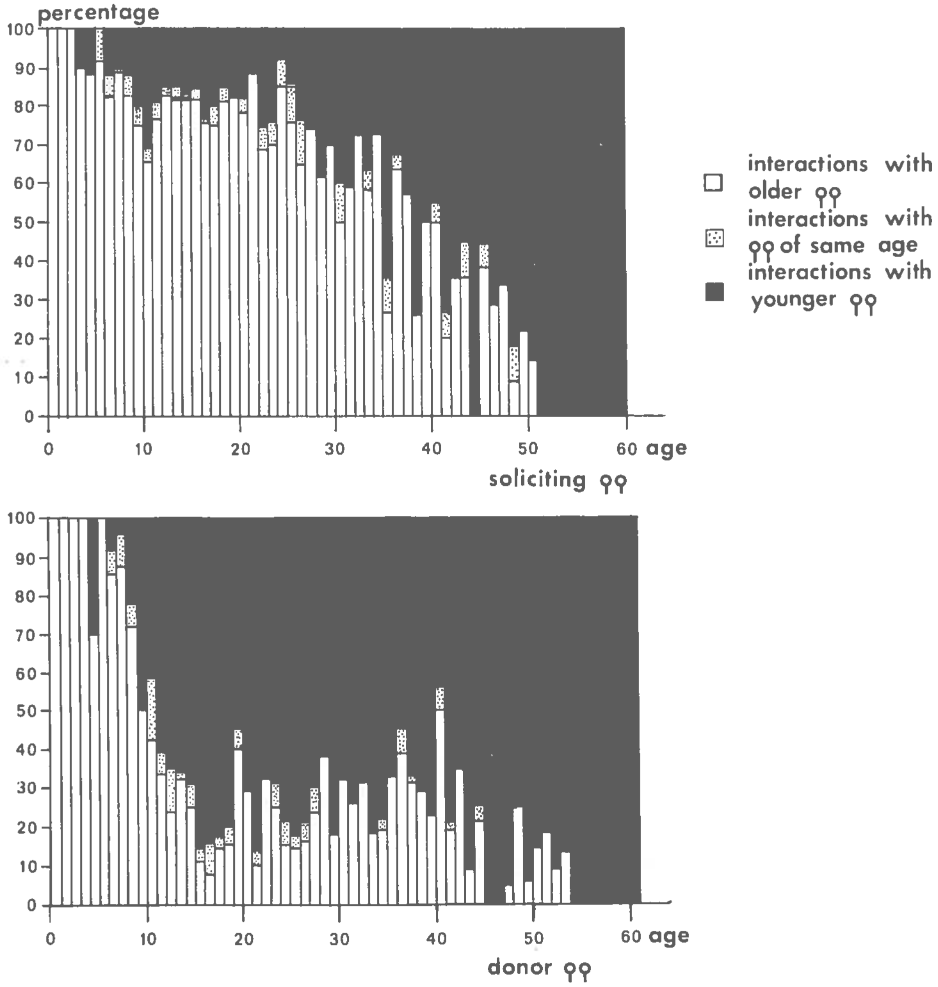


Fig. 4. For bees of the various ages the occurrence of foodtransfers with bees of younger, older, and similar age is shown in percentages. This is shown separately for soliciting bees (above) and for donating bees (below).

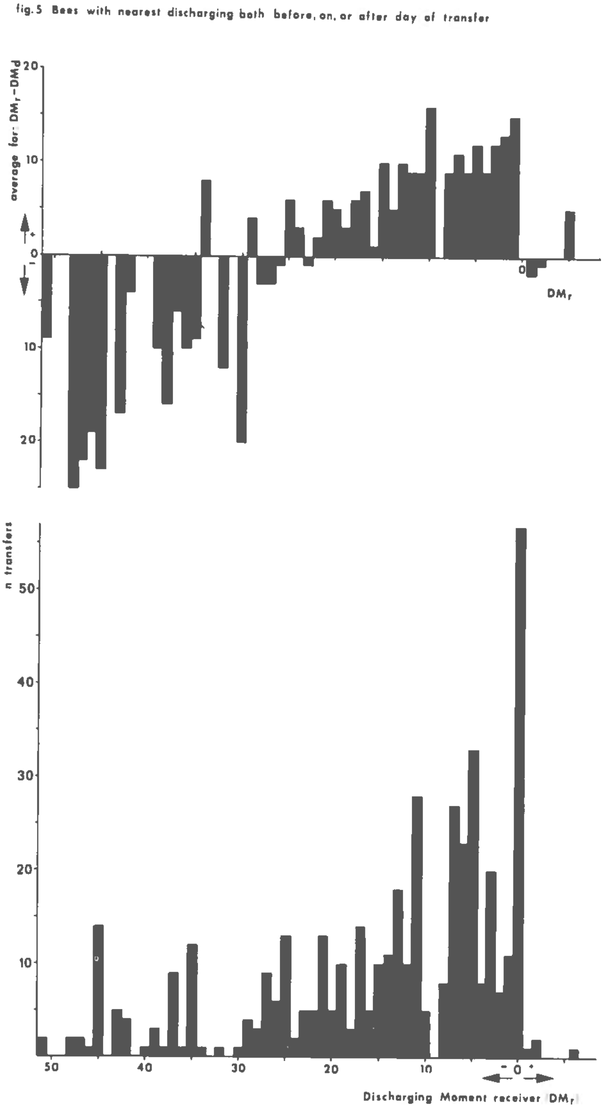


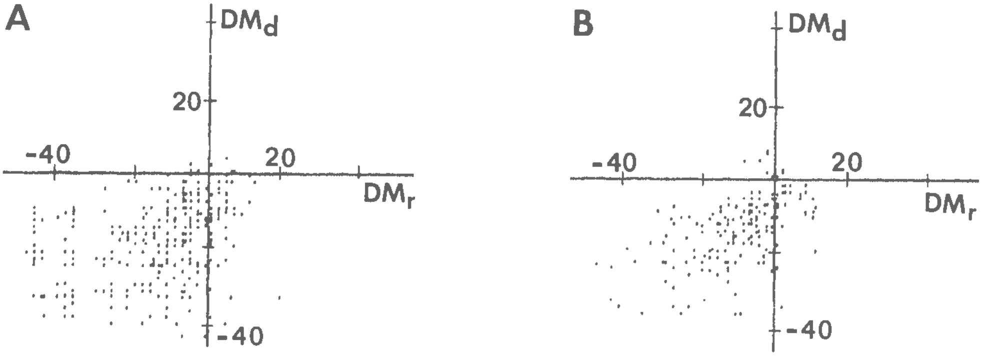
Fig. 5. For all transfers between receivers and donors who both had their nearest discharging either before, on, or after the TC-day, is indicated the average difference between the "Discharging-Moment, DM" of these bees.

solicited bee, was less pronounced in the unrewarded solicitations, although the pattern is not significantly different ($P<0.05$). Soliciting and delivering individuals were compared with respect to the shortest interval (in days) between their participation in a TA and eventual cell provisioning activity.

It appeared that soliciting bees had generally discharged more recently than the donor bees. However, receivers that had discharged more than 25 days previously generally received from donors that had discharged more recently (Fig. 5). Transfers between a receiver and a donor that had both discharged most recently before the TA were common (86%, n=465), as were transfers between bees both discharging on the day of their TA (13%). Transfers between individuals both having their nearest discharge after the TA were rare (1%). A further analysis of the temporal relation of discharging and trophallaxis, both with respect to transfers and unrewarded solicitations, is presented in Fig. 6 and Table 1. From this it appears also that discharging larval food in brood cells is much more related to receiving on that day from bees that do not discharge on that day, than it is related to donating on that day to bees that do not discharge on that day (57% vs. 6%; n=219 transfers). This was similar for the unrewarded solicitations (64% vs. 11%; n=71).

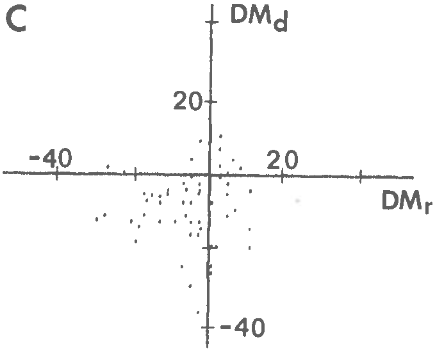
Thus there was strong agreement between the results of these two series: the first in which we registered continuously all occurring POP's and in which we consequently could discriminate TA's of workers for their individual participation in single POP's (cf. our term "immediate dischargers"), and the Trinidad-series, in which we related the nature of a worker's TA to its nearest *day* of discharging brood-food (cf. our term "discharging-moment"). It is evident from both series that the trophallactic behavior of single bees is to a considerable extent determined by their status in the provisioning of brood cells.

During the Trinidad observations one whole day was used for the continuous recording of pollen ingestion from the open storage pots. Returning pollen foragers released their loads in pots other than those from which house-bees took up pollen. It was clear from the distinct decrease of the pollen contents of the pots in which the latter bees worked that they were actually taking pollen from these pots. A total of 687 PF's (pollen feedings) by 154 individual bees were recorded. 88 Of these 154 individuals also participated in larval food discharges during one of the observation days. Most of these



pr.mm.coeff. $r= 0.45$
$p < 0.05$
$n= 424$

pr.mm.coeff. $r= 0.62$
$p < 0.05$
$n= 261$



pr.mm.coeff. $r= 0.35$; $p < 0.05$; $n= 125$

Fig. 6. Relation between shortest interval in days between trophallactic receiving or donating and eventual cell provisioning activity. A + B, For all bees engaged in transfers (for clarity reasons the data are represented in two graphs)*. C, For all bees engaged in unrewarded solicitations*.

*The scores of bees that discharged larval food on the day of their TA are not shown in these graphs; see Table 1.

Table 1. The various trophallaxis behaviors of single bees related to their nearest (previous, present or future) performance of larval food discharges in brood cells.

		Transfers n = 685	Unrewarded solicitations n = 125
A.	same sign for DMr, DMd:	n = 465	n = 54
1.	DMr, DMd < 0	86%	63%
2.	DMr, DMd = 0	13%	26%
3.	DMr, DMd > 0	1%	11%
B.	different sign for DMr, DMd:	n = 220	n = 71
1.	DMr < 0, DMd ≥ 0	6%	10%
2.	DMr = 0, DMd ≠ 0	57%	64%
3.	DMr > 0, DM ≤ 0	37%	26%
C.	different sign for DMd, DMr:		
1.	DMd < 0, DMr ≥ 0	71%	38%
2.	DMd = 0, DMr ≠ 0	6%	11%
3.	DMd > 0, DM ≤ 0	23%	51%

The "Discharging Moment" value with a negative sign (DM < 0) indicates that the nearest discharge took place before the recorded trophallactic act (TA). A positive sign (DM > 0) indicates that the nearest discharging occurred after the TA. DM=0 stands for discharging on the same day as the respective TA. "r", receiver (also used for unrewarded solicitations); "d", donor (also used for unrewarded solicitations).

A. 1. both receiver and donor had their nearest discharging before the TC-day
   2. both receiver and donor discharged on TC-day
   3. both receiver and donor had their nearest discharging after TC-day
B. 1. receiver had nearest discharging before TC-day; donor had this after or on that day
   2. receiver discharged on TC-day; donor had nearest discharging before or after that day
   3. receiver had nearest discharging after TC-day; donor had this before or on that day
C. same TC's as in B, but now analyzed from the donor's side
   1. donor had nearest discharging before TC-day; receiver had this after or on that day
   2. donor discharged on TC-day; receiver had nearest discharging before or after that day
   3. donor had nearest discharging after TC-day; receiver had this before or on that day

bees discharged on days before they acted as pollen feeders (Fig. 7). This is exclusively true for all individuals that were seen more than four times at the pollen pots during the observation day. The two bees most active at the pollen pots, i.e. both observed 27 times, had their observed nearest discharging respectively 9 and 11 days before their PF. In accordance with the results of the first described observation series, the discharging bees did not actively participate in the uptake of pollen from the pots. The reluctance of brood cell provisioners to visit the pollen pots is evidenced by the fact that PF activity is lowest at the age of highest discharging activity (Fig. 7). In this series pollen-feeding bees also belonged to a very wide age group: from bees less than 1 day of age to bees of 49 days old. However, PF was observed most frequently in bees of 14-27 days. Only three of the 18 dischargers seen during the day of continuous observation were encountered among the 154 pollen consumers. Two of these were seen only once at the pots, and one bee only three times. This is a low pollen feeding activity, when compared to that of the other pollen consumers (cf. Fig. 7); it constitutes only 0.7% of all PF acts observed that day. These three bees were respectively 15, 16 and 19 days old. This is rather old for dischargers of *M. favosa* (SOMMEIJER, in press). They had performed most of their discharges on the days before they were recorded as pollen feeders. Two of these bees were recorded as egg layers, 5 and 9 days respectively after the observed PF (Table 2). The five PF recordings of these bees were all, except one, performed after their discharging on the observation day (17/7/79).

Egg-laying by queenright workers is an remarkable trait of stingless bee sociality. *M. favosa* is no exception to this (SOMMEIJER, in press). Therefore, the relation between pollen uptake and worker oviposition appears interesting. However, no detailed observations were directed at the egg-laying activity of the recorded PF bees. Still several of these were seen laying eggs. The two active PF bees that consumed pollen 27 times had laid one egg, respectively 11 and 9 days before. One worker with 12 PF's laid 4 days before that day, and again 3 days afterwards. Some bees had already laid two eggs on days before their recorded PF.

By video-recording TC's during the mass-provisioning of brood cells we obtained detailed information about the trophallaxis of the dischargers during this short behavioral sequence. The recordings revealed that brood cell provisioners also actively solicited during the actual provisioning process. They then often left the cell immediately after their food discharge and started

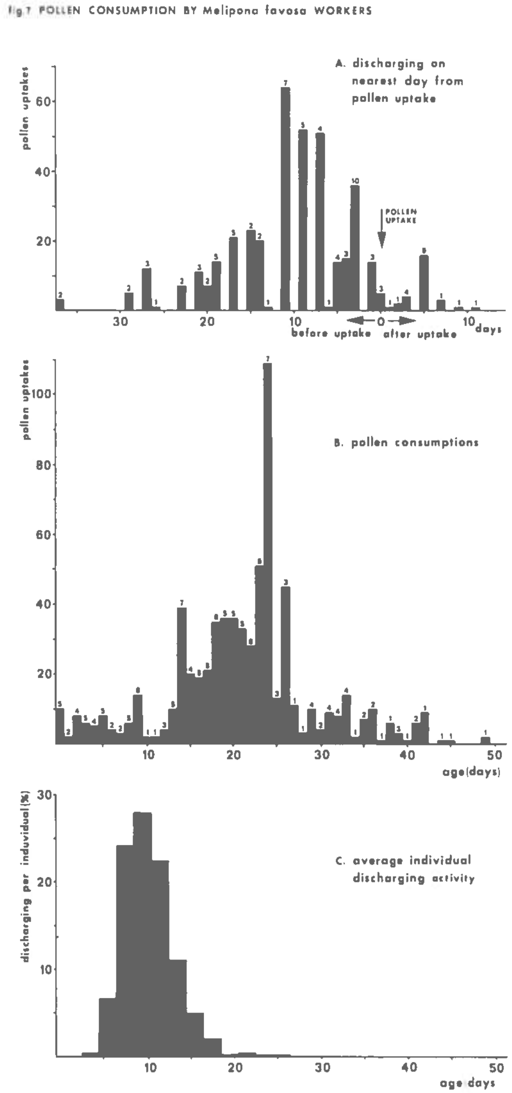


Fig. 7. Pollen consumption in M. favosa workers. Pollen consumption of workers in relation to: A. their nearest day of cell-provisioning before, or after the pollen uptake*; B. the age of the workers; C. the average individual discharging activity for bees of age-groups of two days.

*Since we recorded cell-provisioning principally every other day, the histogram shows alternating high and low values for the consecutive days.

		discharging (participation in number of total observed POP's that day) and oviposition ("ovip") during a series of observation days before and after the pollen feeding observations of 17/7/79													
observation date (July):		10	11	12	13	14	16	17	18	19	20	22	24	25	26
Number of POP's recorded:		6	3	7	2	7	5	3	9	2	6	6	3	5	4
bee code	age at 10/7/79														
mz	7 days	4	3	2	1	4	2	2	1	1	-	ovip	-	-	-
mb	10 days	3	1	2	-	-	-	1	-	-	-	-	-	-	-
ng	6 days	-	-	-	-	-	4	3	2	1	-	-	-	-	ovip

Table 2. Life history of the only three workers that were discharging on the same day as they were pollen feeding, of a total of 154 pollen feeders, on 17-7-79.

	Before IDI	During IDI	After IDI
Times being solicited	1.9 (45)	1.1 (27)	1.3 (32)
Actual donations	0.9 (22)	0.3 (6)	0.5 (16)
Total solicitations	1.8 (40)	8.6 (203)	5.7 (147)
Successful solicitations	0.7 (16)	4.1 (97)	2.3 (59)
Number of workers	104	74	102

Table 3. Trophallaxis of dischargers during POP. Frequency of different trophallactic acts in relation to the "Inter-Discharge Interval, IDI" of discharging workers (Frequency/10 min.; scores between brackets).

active solicitations towards other bees. We regularly observed that these bees returned again as dischargers in the same POP; this was confirmed in numerous direct observations, both in Utrecht and in Trinidad. We studied specifically the trophallaxis behavior of these multiple dischargers in one single POP. We termed the period in between their first and last discharge in a single POP as their "inter-discharge interval, IDI". It appeared that dischargers solicited more during the POP than they were solicited. Their soliciting behavior was particularly intensified just after the discharge. The bees in these recordings with more than one discharge in a single POP (n=74) were, when solicited, reluctant to donate food during their own IDI. These bees not only solicited most actively during their IDI (Table 3), but also their solicitations were then most positively rewarded.

DISCUSSION

The importance of the recent finding by COLE (1981) of an uneven food distribution among the worker dominance hierarchy of *Leptothorax* ants, was emphasized by MAY (1981). This was considered as the first deviation in ants of the generally accepted social stomach principle, which as such was considered as a highlight of eusociality. Our results indicate that the trophallactic network of *M. favosa* does not represent an open social stomach system, leading to an even food distribution. Since the mass-provisioning of brood cells is a very characteristic and general trait of stingless bee sociality, we assume that the trophallactic pattern which we found may be representative for the whole group. In other social insects it has been established that trophic advantage resulting from trophallaxis is related to the reproductive dominance of the queen. In this respect it is important to note that the discharging bees, which were the most "dominant" in the trophallactic system, were not the most active egg layers of that moment. However, these bees did have developed ovaries (SOMMEIJER, in press). It would be of interest to compare the unidirectionality in species with queenright worker-laying and species without such a form of worker oviposition.

It is remarkable that all trophallactic interactions between non-foragers were started by soliciting behavior, and never by a spontaneous offering. This is very different from trophallaxis behavior in honey bees. We also noticed this phenomenon in various other stingless bee colonies. Elsewhere, we have reported the qualitatively different trophallaxis behavior of the foragers (SOMMEIJER, DE ROOY, PUNT & DE BRUIJN, 1983). In a colony of *M. favosa* in Trinidad we found that pollen-returnees successfully begged for food before unloading, while nectar-returnees actively and successfully offered food to nestmates before discharging their nectar load into a storage pot.

It is very unlikely that the bees who take up pollen from the stores deposit this into the brood cells. Pollen as a component of the larval food is, almost certainly, transferred trophallactically to the dischargers in liquid suspension. There is, however, little information about probable qualitative differences in the contents of honey stomachs of the various dischargers. MARTINHO (1975) found that there is less variation in sugar concentration of the honey-stomach contents of stingless bees than is found among the bees of a honeybee colony.

The different dischargers could still add differentially various components of the larval food. A few miscellaneous observations indicated, however, that young dischargers with swollen abdomen always had their honey stomach filled with a pollen-rich suspension.

It should be noted that although PF bees were of all ages, frequent PF was performed only be bees of 14 - 27 days old. Earlier we found that this corresponds precisely to the age-group of the laying workers (SOMMEIJER, in press). The observations on oviposition in this series are further evidence for the fact that laying workers are especially active in PF.

Although larval food dischargers also transferred food to nestmates, they did so exclusively to other dischargers. It appeared that if they donate, this is done mostly to bees that play a more active role in the discharging. For the first observation series we found that 95% of these transfers were made to a "higher classified" discharging bee (classification according to the sequence by which a number of workers discharge in a single POP, cf. SOMMEIJER, BEUVENS & VERBEEK, 1982).

The quick disappearance of a worker from the cell upon discharging was observed by Sakagami and collaborators in several other species. This behavior has been described in these species as the "post discharge escape" (SAKAGAMI, ZUCCHI & PORTUGAL-ARAUJO, 1977; SAKAGAMI, 1982). These authors suggest that the behavior of the discharging bee is governed by three behavioral systems ("drives"): "attack on the queen", "flee away from the queen" and "food discharge". They assume that an abrupt change of the balance between the opposing drives, at the very moment the honey-stomach is emptied, determines the shape of the "escape reaction". In our colonies of *M. favosa*, with individually marked workers, we noted that this "escape reaction" was always strongly shown by those workers who were greatly involved in the construction of a cell, in which they later on, during POP, performed several discharges. Therefore we suggested that this quick withdrawal was not based on fear of the queen (SOMMEIJER, 1979). Now it appears that in between their subsequent food discharges these workers, during one provisioning phase, successfully solicited food from other workers. These observations offer an acceptable alternative for the hypothesis of SAKAGAMI, which has recently been withdrawn (SAKAGAMI, YAMANE & INOUE, in press). After post-discharge withdrawals had also been observed in queenless colonies, e.g. in *Partamona testacea* (SAKAGAMI, BEIG & AKAHIRA, 1964), we also observed their occurrence in queenless colonies of *M. favosa.* Recently these withdrawals were also observed to occur in two species from Sumatra (SAKAGAMI, pers. comm.). They also occur in *Melipona bocandei,* where POP starts in the absence of the queen (SAKAGAMI et al., 1977). Instead of being an escape reaction, this behavior in *M. favosa* may be related to motivation of the respective workers to obtain more food quickly and bring it into the cell. The fact that immediate dischargers receive food mainly from other bees that do not themselves discharge and that dischargers are reluctant to donate, further explains the dischargers' quick disappearance from the cell, because further away from the cell they are more likely to meet less reluctant non-discharging donors.

Finally we conclude that these results indeed show the trophallactic dominance of the temporal cell-provisioners. This evidence for uneven food-transmission in highly eusocial bees (see also: BUTLER, 1974; KORST & VELTHUIS, 1982) together with the result of COLE (1981) for a myrmicine ant, not only requires a reformulation of the social stomach concept, but, in our opinion, it also brings into question the very existence of the assumed random mutual exchange of liquid food in social insect colonies, in which COLE (1981) and MAY (1981) apparently, still firmly believe.

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VIII CELL CONSTRUCTION AND EGG-LAYING IN *TRIGONA NIGRA* VAR. *PAUPERA* PROVANCHER, WITH A NOTE ON THE ADAPTIVE SIGNIFICANCE OF THE TYPICAL OVIPOSITION BEHAVIOUR OF STINGLESS BEES

M.J. Sommeijer, J.L. Houtekamer and W. Bos

SUMMARY

New brood cells of the cluster-building stingless bee, *Trigona nigra* var. *paupera* Provancher, are built in groups. The cells of each group develop into a batch formation when they are provided with larval food and eggs. Oviposition occurs approximately at 24-hour intervals. The cells of a batch are built in a "semi-synchronous" manner. Their provisioning during the very short "integrated oviposition process, IOP" (on the average 3 min 5 sec) is also "semi-synchronous". The behaviour of the queen and the workers during IOP is described. The workers form a very distinct court around the queen exclusively just before IOP. The incessant diving into the cells by workers before and after the discharging of larval food probably has a signalling function for the queen. The queen's exposure of liquid between her mandibles during her final wait at one of the cells immediately before the workers discharge larval food is assumed to represent another form of queen-worker communication.

The ultimate function of the typical rhythmicity of the oviposition behaviour of stingless bees is discussed. This rhythmicity is considered to be an adaptive trait, enhancing the fitness of the queen. We hypothesize that this behavioural feature increases the queen's control over cell provisioning by workers and hence over worker ovipositions. The fact that in many species the queen engages in worker-egg oophagy indicates the importance of oophagy for the expression of queen dominance. This mechanism for the control of male offspring produced by workers is discussed. Under certain conditions the queen may lose full control over laying workers. It is suggested that such limitation of the queen's control mechanism in fact contributes to the "proper" timing of seasonal male production.

INTRODUCTION

This paper deals with the intranidal reproductive behaviour of *Trigona nigra* var. *paupera* Prov. (Apidae; Meliponinae). This species is regarded as belonging to the large *Tetragona* subgenus (WILLE & MICHENER, 1973), but is also considered to be a species of the subgenus *Frieseomelitta* (MOURE, 1961; D.W. ROUBIK & R. ZUCCHI, pers. comm.). It is one of the stingless bees which do not build brood cells in regular combs, but build them in a cluster arrangement. A general description of the architecture of the nest of *T. nigra* is given by MICHENER (1946) and these nests are also discussed and illustrated in the study by WILLE & MICHENER (1973).

Only limited information is available about the intranidal behaviour of cluster builders. BASSINDALE (1955) described this for *Hypotrigona braunsi (=gribodoi)*, and SAKAGAMI & ZUCCHI (1974) reported on *Leurotrigona muelleri* and *Trigonisca duckei*; these species are only distantly related to *T. nigra*. TERADA (1974) studied the production of queens and the ovarial development of the workers in *L. muelleri* and in *Frieseomelitta varia*. The latter belongs to the same subgenus as *T. nigra*. For *F. varia* it was found that queens are reared in specific queen-cells which are larger than normal brood cells, and that the workers even in queenless situations do not develop ovaries. ZUCCHI (pers. comm.) informed us that in the colonies of both *F. varia* and *F. sylvistrii* the queen lays the eggs in single ovipositions. However, colonies of *F. doederleini* produce eggs in batches at regular intervals.*

In this paper we present detailed information about cell construction, cell-provisioning and the egg-laying process. The progress of cell construction was studied along with the behaviour of the queen and workers in relation to this. Specific attention was given to the interacting queen and workers in the formation of a court. The building of brood cells in batches is very common in stingless bees other than *Melipona*. The adaptive significance of this rhythmicity and some related behaviours will be discussed.

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* A detailed account of the various modes of egg-laying rhythmicity is given by SAKAGAMI & ZUCCHI (1974).

Fig. 1. A, cluster of brood cells around the empty bottles in a cardboard beer-bottle crate. B, part of nest in an observation hive in Trinidad. Storage pots at left; brood cells at right.

MATERIAL AND METHODS

T. nigra is a common bee in Trinidad. The species demonstrates a remarkable flexibility for adapting to a wide variety of nesting sites that are available in urban areas in addition to natural sites, such as cavities in trees. In Trinidad it is common to find nests in e.g. fence poles, walls of houses, hollow doors. The original housing of our observation colony, in between the empty bottles in a cardboard beer-bottle crate (Fig. 1), nicely exemplifies this flexibility.

This colony was collected in Las Lomas, Trinidad and transferred to an observation hive on April 5th, 1979. The population was estimated to consist of about 500-700 bees. By the end of April a young laying queen from a conspecific swarm was introduced into the observation hive. The colony was transferred to Utrecht in August, and installed in another observation hive, which consisted of two compartments; the smaller one contained the cluster of brood cells and the storage pots and the other was used for feeding syrup. In order to reach the latter compartment the bees had to pass through a hole (Fig. 2). Pollen food, prepared according to CAMARGO (1976), was introduced directly into the pollen storage cells. There was no exit to the outside.

Most recordings of the behaviour of the workers and the queen were made through direct observation under red light; in addition brood cell provisioning and egg-laying were studied by means of video-recordings. The number of bees in the colony decreased during the progress of the observations from about 200 at the start to about 100 at the end.

Colonies of this species are known for the presence of differently coloured bees in the nest. Young bees are completely pale; gradually the head and thorax darken and finally also the abdomen. In mid-November 1979 the colony consisted of: 5 completely pale bees; 45 bees with head and thorax darkened; and 75 completely black bees.

RESULTS

I Brood nest: general construction

In the shallow observation hive (Fig. 2) the brood cells were built in one round cluster. In the original housing of the beer-bottle crate, the brood

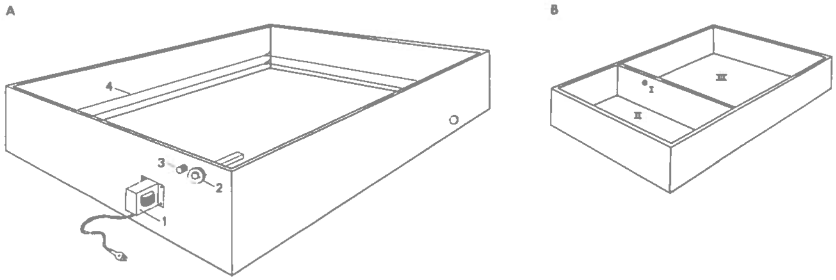


Fig. 2. Observation hive: A, outer-box (60x50x20 cm) with heating device (1, transformator; 2, thermostat; 3, pilot lamp; 4, heating wire); B, inner-box (36x 38x4 cm); I, passage leading from brood compartment (II) to feeding compartment (III). Inner-box and outer-box are both covered with glass plates, when inner-box is placed inside outer-box.

Fig. 3. A close-up of the advancing front, where brood cells are being built on the cluster of sealed cells. Picture taken from above. Brood cells measured 3.0 - 3.5 mm.

nest consisted of clumps of brood cells irregularly arranged around and in between the empty bottles (Fig. 1). The cluster was not very compact. It was a loose arrangement with space between the brood cells, which were connected to each other by short pillars. Sometimes the cells touched each other (Fig. 3). Brood cells measured 3.0-3.5 mm in diameter and about 5.0 mm in length. The storage pots were not always built in the close vicinity of the brood cluster. They had a diameter of about 15-20 mm and were about 30 mm long. The pots sometimes stood on pillars and were often built up against each other.

New brood cells were built simultaneously in groups. The "advancing front", the area of the brood-cluster where the new cells were built (MICHENER, 1974), moved clockwise and spirally around the cluster. In this way the new cells replaced mainly old cells from which bees had emerged. The cluster as a whole was connected to the hive walls by means of pillars which were thicker than those interconnecting the brood cells. As is characteristic for most cluster builders, there was no involucrum surrounding the brood. However, wax plates were built in between the major supporting pillars.

II General activities of groups of bees with different pigmentation

a. Pale workers

Pale workers were always small in number. They were inactive, resting in or on the brood-cluster. When their head had become a brownish colour they engaged in the nibbling of brood cells and in the removal of wax from these. They also participated in the trophallactic food transfers.

b. Half-coloured bees

Half-coloured workers had head and thorax in different stages from brown to black and a pale-coloured abdomen. They spent most of the time on the brood-cluster, but also on the honey storage pots. They were the exclusive builders of brood cells, but also participated in the construction of other structures. They collected the wax scales that had been deposited everywhere in the nest, e.g. on the wooden wall and the glass covers. Wax was also scraped from brood cells. Bees in this pigmentation stage also removed the remains of old cells. They interacted with the queen, provisioned and operculated brood cells. Moreover, they collected pollen from the pollen storage pots. In addition they fragmented dead bees and processed the remains, together with other waste material, into pellets at the waste-dumps.

c. Black bees

Various observations in Trinidad indicated that the black bees are field bees. Within the observation hive, where they had no opportunity to fly out, these bees were engaged in the ventilation of the hive. For this they were positioned in or near the passage leading from the brood to the feeding compartment. The same individuals could be in the ventilation position for periods of up to 15 min. Black bees carried waste pellets from the brood compartment to the feeding compartment. They also worked with a brownish sticky substance, which was deposited in small droplets on the walls of the hive. This material was used for sealing cracks. It was evident that black bees did not build on brood cells nor did they participate in other wax manipulations. They were never observed in interactions with the queen.

III Cell construction

The progress of the construction of brood cells was characterized by a very distinct rhythmicity. Long intervals, in which cell-building took place, alternated with relative short periods of cell-provisioning and egg-laying. These two periods of different duration are termed the "extra-oviposition period" and the "oviposition period" (SAKAGAMI & ZUCCHI, 1974). Discharging and egg-laying in a number of cells (a "batch") during one single oviposition period is called by these authors the "Integrated Oviposition Process, IOP". In *T. nigra* generally all the cells that were built in an extra-oviposition period were provided with food and eggs in the successive oviposition period.

Due to the decline of the colony a diminution in the number of cells per batch from about 12 to 1-2 occurred (Fig. 4). This did not alter the time needed for the completion of a single cell. Also, the IOP's occurred with a constant frequency, namely once per day. Generally, this process occurred at night, after which about 12 hours elapsed before new cells were constructed (an example in Fig. 5). The example of Fig. 5 further illustrates that the various brood cells, which together form one batch, are not built synchronously. The construction of these cells was started at slightly different times, and at a certain moment the cells were at different stages of construction.

However, the building appeared to increase gradually in intensity, and cells that were started later were built more quickly. It did happen, however, that the construction of a certain cell had not progressed far enough at the

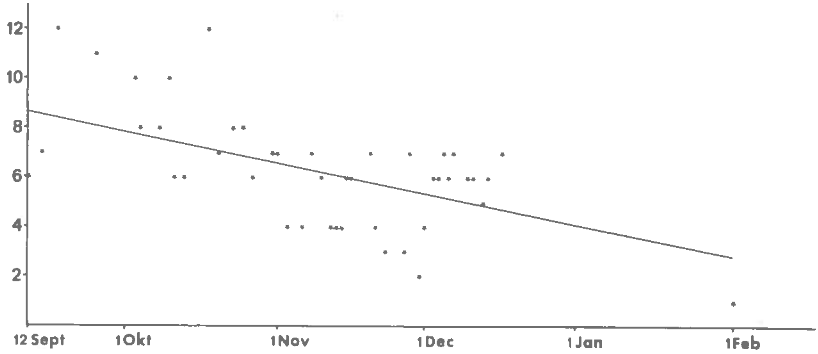
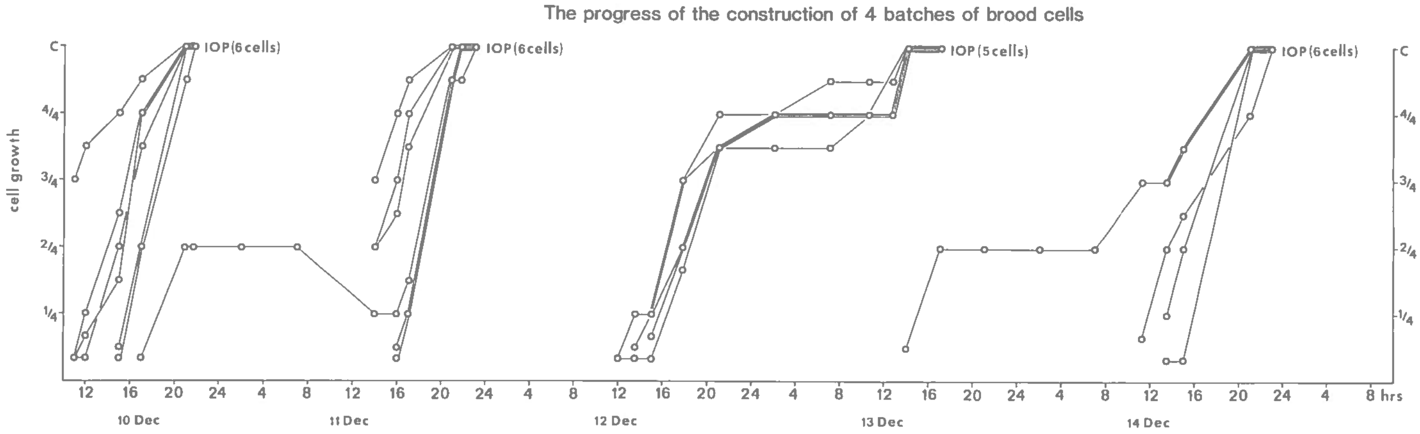


Fig. 4. Number of cells included in the various batches during the total period of observation.



The progress of the construction of 4 batches of brood cells

Fig. 5. The progress of the construction of the various cells of four batches is shown. The construction of all cells was registered separately by means of hourly recordings in the daytime, and two-hourly or three-hourly recordings at night. As construction stages we discriminated the respective four quarters of the height of a sealed cell, and the additional stage of a complete collar (c). The numbers of cells included in the IOP's are indicated between brackets. The final cell of the last batch was built late and very fast. Since we only observed this cell in the final construction stage, just before IOP, it is not shown with a line.

start of the IOP for the cell to be included in this. The building of such a cell was then discontinued for a considerable time. It also occurred that such a cell was even partly demolished, after which it was normally incorporated in the new group of cells (cf. Fig. 5). It sometimes happened (as is shown for the third IOP in Fig. 5) that the building of the group of cells which had already progressed considerably came to a standstill. Such delays lasted about 24 hours. It also happened once or twice that, because of a delay of 12 hours, the cycle with IOP at night switched to a rhythm of 24 hours with IOP in the daytime, which was maintained for a period of up to a few weeks. The incidence of such delays was not higher at the end of the observation period. In general the IOP takes place shortly after all the cells of the batch are provided with smooth collars. The diurnal rhythm therefore pertains to the construction rather than to provisioning.

IV The integrated oviposition process

During the cell construction process there was an increase in the building activity. In the last period before the start of the IOP, the cells were hardly ever left unoccupied. Workers then even waited for each other at the side of a cell for an opportunity to insert their body into the cell. The body-insertions of cell-constructing bees became shorter, and they alternated more frequently. As a result, these insertions became short dives into the cells.

The queen, who remained away from the advancing front, and generally away from the cluster during most of the extra-oviposition period, became more active towards the end of this period. The frequency and the duration of her visits to the advancing front increased. The court of workers (Fig. 6) surrounding her as soon as she halted during her patrolling through the nest was very distinct in this phase. After cruising through the advancing front the queen remained ("waiting") at one of the cells of the batch. The gradual change from "cruising" to "waiting" was in accordance with the basic pattern described by SAKAGAMI & ZUCCHI (1974).

The queen moved once or twice from cell to cell before remaining at the final place. During her "final waiting" (cf. SAKAGAMI & ZUCCHI, 1974) she hung on to the walls of this cell, in such a way that the workers could continue diving alternately into the cell. At this stage the queen kept her wings spread out without vibrating them. She then performed incessant rhythmical movements with her mandibles open. We clearly observed the presence of a liquid substance between these; this liquid was continuously stirred by the movements of the mandibles. In the meantime she moved her antennae slowly. She could advance from this position for a short insertion of head and thorax into the cell or she could turn sidewards for an interaction with one of the workers who were concentrated around this cell. There were also worker concentrations around other cells of the batch, but the concentration was most pronounced around the cell with the fixated queen. Sometimes a worker who had mounted the cell, suddenly advanced towards the fixated queen with raised-forebody and opened mandibles. This form of "cell-covering" is also described for *Trigonisca* by SAKAGAMI & ZUCCHI (1974). The queen of *T. nigra* often reacted to this by a very short withdrawal, immediately followed by an advance towards this worker who may be touched with antennae, or sometimes, with the front legs. The very distinct ruffle beatings described for *Melipona* in the same context did not occur, nor did other forms of intense body-contact.

Around this time the first discharge of larval food by one of the workers occurred. The abdomen of a discharging worker "telescoped" to less than half its normal length. By this contraction the crop was emptied. Immediately after this first discharge other bees began to discharge. Only a few contractions sufficed for the filling of a cell with liquid larval food for to about two-thirds of its capacity. The average number of discharges per cell was 3.5 (SD=0.6) ranging from 2-5 (n=74 cells).

Shortly after the start of provisioning in the first cell, the other cells were filled also, so the discharges in various cells were partly synchronous. It is noteworthy that the first discharges were not always in the cell where the queen was waiting. The chronological order in which the respective cells of 11 different batches were provided with food and eggs is shown in Fig. 7. The position of the queen at the start of provisioning is also shown in this figure.

Once the cell was provided with the necessary amount of food the workers continued to perform head insertions. This was continued until the queen approached. Generally before laying she also inserted her head into the cell. We assumed that she ingested some of the larval food. After this, she oviposited, withdrew from this cell, actively walking through the cluster until she reached another cell that had been filled. Here she repeated her head insertion and the oviposition in the same manner. If she reached a cell in

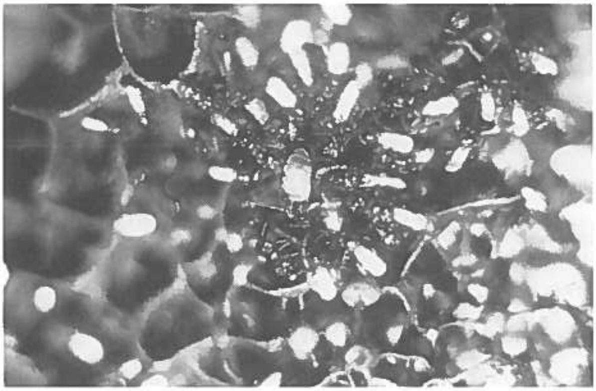


Fig. 6. Configuration of the court around the queen when she is at the brood cluster shortly before IOP. She is surrounded by about 15 workers.

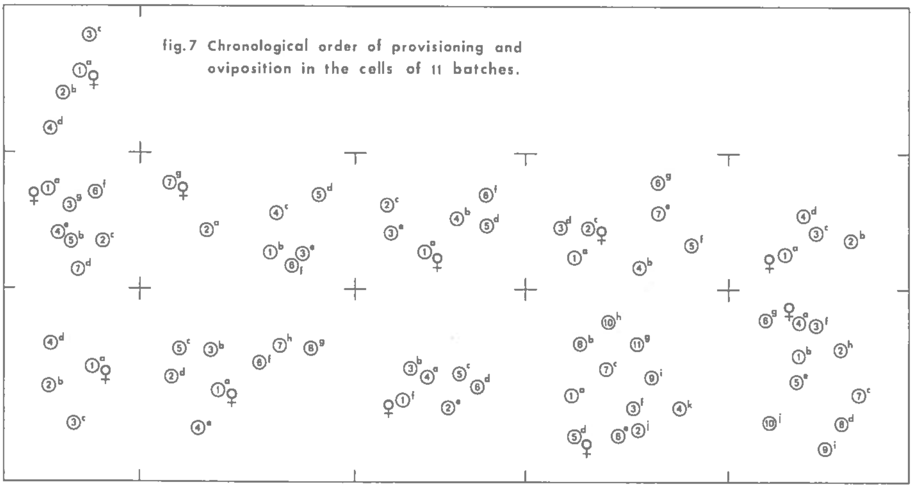


Fig. 7. Chronological order of provisioning and egg-laying in the various cells of 11 batches. The cell where the queen waited before the first discharge is indicated (♀). Numerals indicate order of provisioning; letters indicate order of oviposition. The spatial arrangement of the cells, seen from above, is schematized by the position of the circles. For details of behavioral sequence during one IOP see: Fig. 8. For measurements of cells and their interdistance see: Fig. 3.

which the workers were still discharging, she either waited here for the process to terminate or proceeded to another cell that was already fully provisioned. Her high-speed walking through the advancing front resembled an undirected random route. During this walking she vibrated her wings intensively.

Once all provisioned cells were provided with an egg, she continued cruising for considerable time. We never saw her missing a provisioned cell. Although the operculation by a worker generally followed immediately upon oviposition by the queen, this was sometimes delayed for periods ranging from 4-23 seconds. This operculation was very different from the common pivoting movements of rotating workers in *Melipona*. The operculating worker pushed the collar inwards by folding it in about four flaps. Only when the cell was closed except for a small central hole may a second worker join in the termination of the operculation. The detailed chronological behaviour of queen and workers during the course of one IOP is shown in Fig. 8.

It seems of interest to note that after releasing the contents of its honey-stomach, the bee withdrew from the cell and generally disappeared from the advancing front. Only on a few occasions we did observe that a certain bee discharged more than once. This happened when a bee, apparently ready to discharge, approached a cell which was already nearly filled by two or three other workers. After a slight abdominal contraction in this cell, the worker then proceeded immediately to another cell where a full contraction followed.

The duration of IOP was measured from the start of the "initiating" food release to the start of the final operculation. For 11 IOP's we measured an average duration of 3 minutes and 23 seconds (SD=1 min, 20 sec). The continuation of the queen's cruising through the advancing front could, however, last for a period that was about four times as long. She then gradually extended the area in which she performed this apparant random cruising, for some minutes. However, soon she became less active and remained in this state until the next oviposition period started.

It should be noted that we never observed an oviposition by a worker. This is very different from *Melipona* and many other stingless bees where worker ovipositions occur frequently.

fig.8 Progress of behavior during one IOP

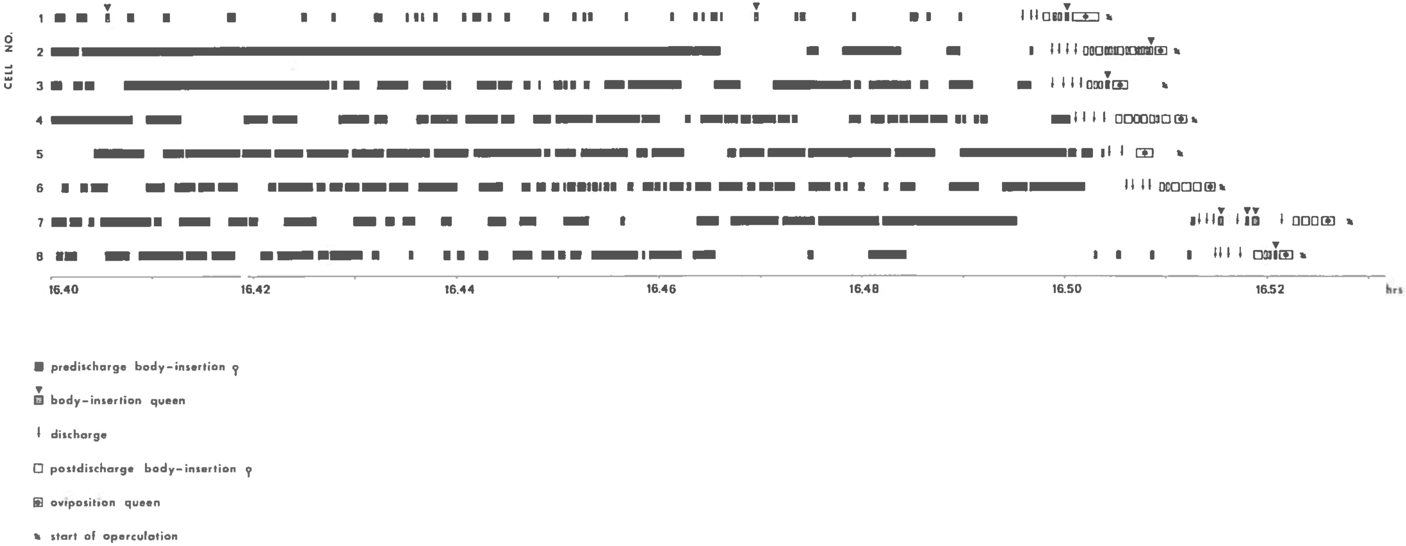


Fig. 8. The progress of the behaviour of queen and workers is schematized for one IOP. The sequences of behaviour taking place at each of the 6 cells of the batch are indicated separately. The cells are illustrated in the order by which their provisioning started. In addition to this sequence is shown how the queen moves from cell to cell (by symbols for her body-insertions and ovipositions). Further information relates to: frequency and duration of body-insertions by workers (before and after provisioning), number of discharges, duration of oviposition, and moment that the operculation started. (This is second IOP from left bottom in Fig. 7)

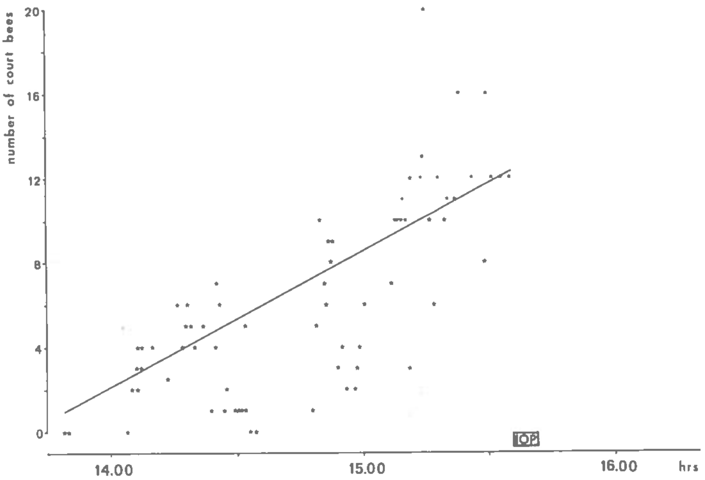
V Interactions between queen and workers

Most interactions between the queen and workers were performed in a configuration that resembled the royal-court of the honeybees. While the queen during most of the extra-oviposition period was resting at some distance from the advancing front either in the cluster or on the floor of the hive she could be attended by a few workers. During her resting the queen regularly performed typical leg-passings over her body and specific vibrating movements with her wings. These behaviours and those of the courting workers were basically similar to those described for other stingless bees (review by SAKAGAMI, 1982). Court workers performed incessant "rocking movements" and now and then sudden advances towards the queen ("dartings").

The resting queen stretched her antennae when a worker approached her and turned towards a worker who performed "rocking movements" or "dartings" at her side. She nearly always seeked oral contact with such a worker. With her antennae and extended glossa she touched the face and head appendages of the worker, and even grasped the worker's head laterally with her front legs. Sometimes the glossa of the queen was between the mandibles of the worker, but never for longer than two seconds, and we had the impression that a transmission of food occurred only rarely. Nevertheless we interpreted this behaviour as food-solicitation. While queen and worker made this buccal contact both animals often lifted their fore-legs and touched each other with their fore-tarsi (described as "hand-shaking" in *Leurotrigona*, SAKAGAMI & ZUCCHI, 1974).

The very weak or sometimes even absent court during the first part of the extra-oviposition period gradually developed into a very distinct court at the time when the brood cells were being finalized. In this period the queen was more active and left her resting place regularly. When she then halted during her tours on the brood cluster she was surrounded by a large number of workers (Fig. 6). Some court bees raised their fore bodies briefly, lifting their front legs and opening their mandibles against the queen. This drastic increase in the number of bees in the court is shown in Fig. 9. It should be noted that there was no court behaviour immediately preceding the IOP, when the queen was "finally waiting" at one of the cells of the batch. The complete absence of court behaviour immediately after the termination of an IOP, although the queen may still be present at the advancing front, is also very remarkable. This behaviour showed clearly that the distinctly fluctuafig.9 increase of court bees

A. IOP on 1Nov 1979



B. IOP on 15 Nov 1979

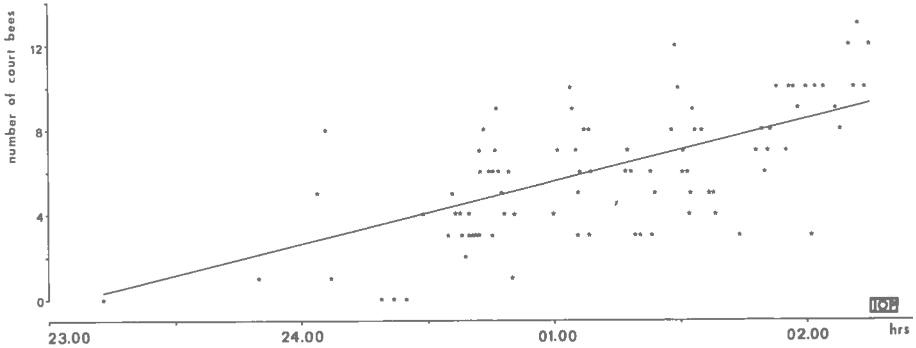


Fig. 9. The number of bees present in the court at different times before the start of two IOP's is shown. There is a distinct increase of the size of the court. There is no court activity during IOP and shortly after this.

A. pr.mm.coeff. r=0.75; n=69; $p < 0.005$

B. pr.mm.coeff. r=0.65; n=88; $p < 0.005$

ting attention of the workers for the queen was synchronous with the oviposition rhythmicity.

DISCUSSION

On the basis of their important serial studies on stingless bee egg-laying behaviour SAKAGAMI & ZUCCHI (1974) have classified comprehensively the different modes of cell construction and larval food provisioning. *T. nigra* can be conveniently fitted into this system. In the terminology of these authors we can conclude that *T. nigra* is behaviourally characterized for the cell construction: as "semi-synchronous", for the formation of the batches: "exclusively batched" and for the serial performance of food discharges in the cells of the batch: "semi-synchronous".

The rhythmicity of the general pattern of oviposition behaviour was pronounced. The complete process of cell-provisioning and egg-laying was carried out in just about three minutes, whereas these short sequences occur only about once every 24 hours. The construction of the cells of a batch took much less than 24 hours (e.g. Fig. 5).

The delay in the start of cell building, after the termination of an IOP, is still difficult to explain. From our miscellaneous observations in Trinidad and from the fact that the related species *F. doederleini* demonstrates a similar frequency of batch formation (ZUCCHI, pers. comm.), we can rule out the possibility that this delay was an artefact. Comparison of *T. nigra* with other species may indicate a relation with other behavioural characteristics of *T. nigra*, e.g. the low frequency of food transmissions combined with the large capacity of the workers' honey-stomach (inferred from the extended abdomen and the few food releases needed to fill a brood cell). In *Melipona favosa* we have seen (SOMMEIJER, DE BRUIJN & VAN DE GUCHTE, in press) that the individuals that provision cells with larval food obtained this liquid food suspension trophallactically from other workers. The latter workers collected the pollen for this food from the food pots. The active trophallaxis of the temporally discharging bees enabled these workers of *M. favosa* to perform several discharges in a few successive cells, or even to carry out more than one food release in a single cell. In *M. favosa* there are about 11 discharges per cell. It could be that *T. nigra* nurses need the long delays to collect, and possibly process, by themselves a sufficient amount of larval food to be able to discharge. As in other species with distinctly extended abdomens and few food releases per cell, the *T. nigra* nurses do not lay eggs.

Although we have no quantitative data on the correlation between these behaviours at the level of individual *T. nigra* bees, the rhythmicity of court behaviour is synchronous with that of cell building. This is similar to the relation between cell building and court behaviour in *M. favosa.*

The rhythmicity of the formation of batches did not change much during observations over several months. However, the number of cells included in the batches distinctly declined. From this finding, and from the very distinct occurrence of court behaviour shortly before IOP, we can conclude that the start of the provisioning and oviposition process is probably to a greater extent induced by the behaviour of the courting workers than by a revived egg-laying motivation of the queen. The queen probably had a capacity for laying more eggs than she actually did in the observed IOP's. She always cruised through the advancing front for a relatively long time after she had oviposited in the final cell. In our study of court behaviour in *M. favosa* we found a strong indication of a regulatory effect of this behaviour for the locomotion of the queen. For *M. favosa* we concluded that the gradually increasing court activated the queen to leave her resting place and to move towards the edge of the new combs. The provisioning can start when the queen arrives at the ready cell (SOMMEIJER, 1982). It seems probable that a similar mechanism functions in *T. nigra.*

The quick withdrawal from the cell by bees that have discharged is a distinct characteristic in most species of stingless bees. This behaviour was called "post-discharge escape" by Sakagami, Zucchi and other collaborators. Sakagami first considered this as an escape from the queen. We associated such quick withdrawals of *M. favosa* with the workers' motivation to bring quickly more food into the cell (SOMMEIJER, 1979; SOMMEIJER, DE BRUIJN & VAN DE GUCHTE, in press). SAKAGAMI changed his term to "post-discharge removal" after these withdrawals had also been found in queenless colonies of two species from Sumatra (SAKAGAMI, YAMANE & INOUE, 1983 and pers. comm.). In *T. nigra* it is evident that the quickly withdrawing workers are not escaping from the queen herself, since they also withdraw in the same manner from cells at some distance from the queen.

According to SAKAGAMI & ZUCCHI (1974) the brief insertions of head and thorax performed alternately by various bees just before the final fixation of the queen at one of the cells are intentional food discharges. In earlier publications these authors termed this behaviour "prefixation excitement". This term indicates the sequential occurrence of the behaviour of the workers and the queen. In other words the workers already perform this behaviour before the queen finally arrives ("fixates") at the cell.

We hypothesize that the communicative function of this behaviour is to indicate to the queen the location of the cell to be serviced. We observed the striking sequential occurrence of first incessant body insertions by workers followed by the arrival and subsequent fixation of the queen only at this cell, first in *M. favosa* and *M. scutellaris trinitatis*. The present study revealed that this behaviour is also very pronounced in *T. nigra*. However, in this species this behaviour is also performed at another time. As soon as the various cells of the batch are filled by the characteristic small number of discharges, the workers proceed to carry out the rapidly alternating body insertions again, but now in the provisioned cell. The communicative function, which has been attributed to this behaviour in the prefixation stage, could also be attributed here. At this stage this behaviour directs the queen to cells that are already filled. Such signalling can be considered as most important here. The various cells of the batch are rather irregularly positioned in the advancing front. Besides, they are not all filled at the same time. In order to find the provisioned cells, the queen has to cruise several times through the area of the advancing front. We can assume that her search is facilitated by the "signalling" of the rapidly inserting workers.

THE ADAPTIVE SIGNIFICANCE OF THE RHYTHMICITY OF THE OVIPOSITION PROCESS IN STINGLESS BEES

The rhythmicity of oviposition behaviour is very characteristic for all stingless bees. This phenomenon is equally distinct in species with low oviposition frequency and species which produce large numbers of eggs daily. In the latter species, which occur in very populous colonies, the egg-laying rhythmicity involves batches of brood cells that are serviced as a group at the same time.

It should be noted that exept for some species, e.g. *T. nigra* and *F.*

varia (TERADA, 1974), stingless bees have another very characteristic trait with respect of oviposition behaviour, namely the very wide spread occurrence of oviposition by queenright workers.

We suggest that the typical rhythmicity of the oviposition behaviour in stingless bees should be interpreted as a trait adapted to enhance the relative reproductive success of the queen in comparison with that of the workers, which in principle oviposit as well. This consideration can be explained as follows.

a. Reproductive dominance by means of oophagy

For all species described (except for *Meliponula bocandei*, SAKAGAMI, ZUCCHI & PORTUGAL-ARAUJO, 1977) it has been reported that the provisioning of the brood cells under queenright conditions can only take place after the arrival of the queen at the ready cell. Her arrival at the cell is usually followed by specific queen-worker interactions (e.g. tappings by the queen on bodies of inserting workers). The very fact that the actual discharging can take place only after these interactions and/or after the "cell-inspection" by the queen indicates that the queen controls the provisioning and hence the oviposition by workers (in principle, oviposition takes place in provisioned cells). Queen oophagy immediately after worker oviposition, examplified by many *Melipona* spp., indicates how this control is basically effected.

b. The evolution of batched ovipositions

It is important to note that the colonies of *Melipona* spp., where cells are built successively and predominantly oviposited singularly, namely as soon as they are ready, are composed of relatively small numbers of bees. In the species with more populous colonies and consequently higher oviposition rates, the ovipositions are always grouped in time.

In our opinion this batched type of oviposition gives large advantages to the queen in relation to her control over worker ovipositions, in contrast to a situation where a similar large number of cells would have to be serviced singularly. The very fact that in the investigated species where worker ovipositions occur, the workers who construct and subsequently provision the brood cells are those with developed ovaries implies a severe menace to the queen (SOMMEIJER, BEUVENS & VERBEEK, 1982). The only way for the queen to exercise firm control over the reproductive activity of these workers is to exert a rigid regulatory effect on some stage of the behavioural cycle of cell building, provisioning, laying, operculation. We consider that the start of cell provisioning has evolved as the regulatory target in this respect. However, if large numbers of cells are built successively, and if these are serviced singularly as in e.g. *Melipona favosa,* this control mechanism will not suffice any more; or at least it will prove very inefficient.

Our view has been influenced by the following observations. Very often we noticed that it took a relatively long time for the queen to arrive at the comb after "prefixation excitement" had started. During the queen's prolonged absence, the workers continued body insertions for some time, but finally stopped this activity if the queen did not appear. In a recently arrived colony of *Melipona scutellaris trinitatis* we observed on one occasion that after a prolonged absence of the queen during prefixation excitement the workers did finally start the provisioning before she was on the comb. The provisioning was performed normally, and a worker subsequently oviposited. The same worker also started the operculation immediately. However, the queen did arrive at the comb just prior to the final closure of the cell. Most interesting was the fact that she herself then opened the cell with her mandibles. Subsequently, she ate the worker's egg and mounted the cell for her own oviposition. Her oviposition, however, failed, she dismounted to turn around the cell. In the meantime another worker mounted the cell and oviposited. This worker succeeded in the complete operculation of the cell. This time the queen demonstrated much less interference with the operculating bee than the first time. This supports our view that queen's control over worker ovipositions is based on a delicate communication and dominance system. This system must be less reliable when single ovipositions follow each other continuously particularly if they moreover occur at different places in the broodnest. The problems that the queen then faces can clearly be appreciated when the brood-nest of a strong thriving colony of *M. scutellaris trinitatis* is observed. In such a colony a large number of cells under construction can be found at the edges of various combs.

We assume that the evolution of batched ovipositions in species with populous colonies promotes control by the queen. The convenient positional arrangement of the cells, as well as the servicing concentrated in the time, make it easier for the queen to survey and exert control over worker ovipositions. From the sociobiological point of view it is conceivable, or even imperative, that the workers again try to escape this control. In certain species we do indeed find that worker oviposition has evolved to the laying of two types of worker eggs. The first type, a specific trophic egg, is "offered" to the queen; the second type, a viable male-producing egg, is laid after the queen's oviposition.

c. Possible regulation of male production

On the basis of the above considerations, an interesting assumption can be made concerning the regulation of the production of males. Very little is known about this matter. BEIG (1972) was the first to find that in *Scaptotrigona postica* workers may play an important role in the production of males. Further observations on the production of males in this species were made by BEGO (1982). We have observed that queenless colonies of various *Melipona* spp. can produce large numbers of males (see also: SOMMEIJER & VELTHUIS, 1977). The limitations of the suggested behavioural control by the queen over worker ovipositions described above may in fact represent the regulatory mechanism itself. In strongly expanding colonies the control by the queen is weakened specifically at temporary increasing oviposition rates. The then partly uncontrolled worker ovipositions may thus result in the production of males. In this way the colony could react adequately to environmental variations.

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SUMMARY

Many aspects of the social behaviour of the stingless bees (Apidae, Meliponinae) are still incompletely known. The purpose of the studies reported in this thesis was to obtain more insight into the basis of the social organization of these bees. Particular emphasis was given to the behaviour of individual bees. Most studies were performed with colonies of *Melipona favosa* from Surinam and Trinidad. The behaviour of the workers was studied after marking all the individuals of the colony. First of all a whole colony was marked in one single operation. Later this technique was improved so that we were able to mark all bees of a hive individually as they emerged from the cells. The information thus obtained relates to various aspects of the intranidal behaviour of these bees. First we found that the bees who do most of the building of a particular brood cell are also the most active in providing this cell with larval food. The age-dependence of these and other behaviours was then studied. Bees that construct and provision brood cells are found to be in the process of developing their ovaries. They oviposit at the end of the period in which they perform nursing activities. The provisioning workers are very dominant in the trophallactic network of the colony. The fact that they receive liquid food from other workers even enables them to perform several discharges of larval food into the same brood cell.

The queen-worker interactions, especially the behaviours of court-workers, were studied in detail. For most of the time the queen stays away from the brood cells under construction. Only when the cells are ready does she visit this part of the nest. This is in proper time for her to "co-operate" in the initiation of the worker discharges of larval food into the cell. The complete provisioning of the cell takes only a minute or two, and is immediately followed by the oviposition and operculation. A strong correlation was found between the behaviour of an individual bee engaged in cell construction and provisioning, and the participation of this bee in court-behaviours around the resting queen. Such a bee shuttles frequently between the "advancing-front", where it actively, in alternation with others builds on brood cells, and the distant queen, in whose court it actively performs specific court acts.

The number of these court workers and of their court acts increases during the period while cells are being prepared. This is followed by the

departure of the queen from her resting place when the cells are nearing completion. We conclude that the typical court-behaviour, which still contains various antagonistic behavioural elements and which in other stingless bees has evolved into very ritualized "bee-dances", is of importance for the regulation of the typical locomotive pattern of the queen. As such, the queen is thereby "informed" about the state of the cell construction.

The typical rhythmicity of the reproductive behaviour of stingless bees is well known but poorly understood. A hypothesis concerning the adaptive significance of this behavioural rhythmicity has been developed on the basis of our observation of the court behaviour of active cell provisioners. These bees were found to have developed ovaries and to engage frequently in egg-laying. The queen of *M. favosa*, like the queen of many other species, exerts her control over worker reproduction by means of oophagy. By controlling the start of the cell provisioning she ensures that worker eggs can only be laid in her presence. By eating these, she subsequently eliminates worker offspring. The evolution of batched ovipositions in species with large nests is discussed in the light of this form of oophagy. It is also suggested that the limitations of such a mechanism of queen control may ensure the "proper timing" of seasonal production of males by workers.

The most recent finding included in this thesis concerns the laying of worker eggs that are morphologically different depending on the presence or absence of the queen. Stingless bee queens appear to be able to exert control over the quality, and hence the viability, of the worker egg; this is a hitherto unknown phenomenon of queen dominance in social bees.

It is concluded that the social organization of stingless bees is basically different from that of honeybees. Within their general social patterns, however, the 300 or so species of stingless bees contain a wide variety of alternatives for the social regulation of reproductive behaviour within the nest. Through this diversity they provide rich material for comparative studies of social behaviour.

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CURRICULUM VITAE

De schrijver van dit proefschrift is geboren op 28 november 1944 te Ovezande. In 1964 behaalde hij het diploma HBS-b aan het Christelijk Lyceum voor Zeeland te Goes.

In dat jaar werd begonnen met de studie biologie aan de Rijksuniversiteit te Utrecht. De doktoraal studie omvatte de hoofdrichting vergelijkende fysiologie en de bijvakken toegepaste entomologie en histologie. Er werden onderwerpen bewerkt op het Laboratorium voor Vergelijkende Fysiologie en het Zoölogische Laboratorium te Utrecht, het Laboratorium voor Entomologie te Wageningen en proefboomgaard "De Schuilenburg TNO" te Lienden. In de periode van 1967-1970 werden student-assistenschappen vervuld in Utrecht en Wageningen. Bij het doktoraaleksamen in februari 1971 werd tevens de onderwijsbevoegdheid verkregen.

Van 1971-1974 was hij als entomoloog van FAO/UNDP (assistent-deskundige) werkzaam voor de Caribbean Plant Protection Commission (CPPC) in Trinidad en Tobago. Dit werk betrof o.a. onderzoek naar de verspreiding van insektenplagen in het Caraïbisch gebied en training van gewasbeschermingsambtenaren in CPPC lidstaten. Van 1974-1976 was hij als entomoloog werkzaam in het FAO/UNDP projekt in Nicaragua voor de ontwikkeling van een geïntegreerde insektenbestrijding bij de kleinschalige teelt van lokale voedselgewassen. Hij was docent aan de Universidad Autonoma de Nicaragua te Managua en León en aan de Escuela Nacional de Agricultura y Ganaderia te Managua. Sinds september 1976 is hij als wetenschappelijk medewerker verbonden aan het Laboratorium voor Vergelijkende Fysiologie van de Rijksuniversiteit Utrecht.