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Simultaneous queen raising and egg laying by workers in Africanized honeybee colonies (*Apis mellifera* L) in Costa Rica

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Summary — Twenty small queenless colonies of mixed European-Africanized origin were set up to study the development of emergency queen cells, ovarian activation and egg laying by workers. In 2 of the colonies, laying workers were present in large numbers before the queens matured from the emergency queen cells. A low number of worker-laid eggs was found in 7 colonies. In the other colonies, the level of ovarian activation of the workers at the end of the queenless period was very variable. We conclude that the occurrence of egg-laying workers in colonies that still contain young brood is not a rare phenomenon in Africanized bees. No worker-laid eggs were found in any of the colonies once a queen had emerged. There was no correlation between the level of ovarian activation (*ie* the size of the eggs inside the ovaries) and either the number of queen cells raised or the number of empty queen cups constructed. The average number of ovarioles per worker per colony varied between 3.79 and 8.33 per ovary. Since there was no correlation between the number of ovarioles and the level of ovarian activation (either within or between colonies), we conclude that these are independent traits in a mixed population.

Africanized honeybees / worker reproduction / population biology / ovarian activation / ovarioles

INTRODUCTION

The appearance of laying workers in *Apis mellifera* is usually presumed to be restricted to queenless colonies which do not contain young brood from which a queen can be reared (Winston, 1987; Moritz and Southwick, 1992). Workers do not generally seem to lay large numbers of eggs before a colony has become 'hopelessly queenless' (*ie* without the opportunity to produce queens). However, van der Blom and Arce (1991) presented a number of incidental observations of large numbers of laying workers in

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Africanized colonies that were simultaneously rearing queens (*ie* within a week after these colonies became queenless).

The Africanized honeybees are considered to be New World descendants of an African subspecies, *A m scutellata*. Until 1982, when the first Africanized honeybee swarms arrived, beekeepers in Costa Rica used to work with bees of mixed European origin, probably mostly related to *A m ligustica* (for reviews concerning the ecology of Africanized honeybees, see Otis (1982) Roubik (1989) and Winston (1992)). Ruttner and Hesse (1981) found egg-laying workers in colonies of *A m scutellata* 7–13 d after the colonies had become queenless (average 9.5 d), which is a much shorter period than was found for colonies of 4 European races (from 16 to 30 d). They tested ovarian activation in workers from 'hopelessly queenless' colonies of different races of honeybees. Hellmich *et al* (1986) show that in mixed queenless colonies (without brood) of Africanized and European honeybees, reproduction is almost exclusively performed by the Africanized workers.

The aim of this paper is to investigate the occurrence of laying workers in colonies which are still raising queens. Different characters have to be studied to quantify the potential for worker reproduction: 1) the actual laying of eggs; 2) ovary activation, *ie* the presence of eggs in qualitatively different developmental stages within the ovarioles; and 3) the number of ovarioles per ovary. The latter morphological character differs considerably among *A mellifera* races (Ruttner and Hesse, 1981). It may be presumed that the number of ovarioles morphologically determines the potential quantitative reproductive capacity of workers (Velthuis, 1970; Velthuis *et al*, 1971), although the activation of this system in individual workers is determined by social factors (Korst and Velthuis, 1982; van der Blom, 1991).

MATERIALS AND METHODS

This study was performed in San Isidro del General, Costa Rica. Twenty full-size queenright colonies were obtained from commercial apiaries from which small queenless colonies were derived. All these experimental colonies were housed in Langstroth hives with 4 frames, 1 containing brood (in all stages, but with a large percentage of young brood), 1 with honey and pollen and 2 empty frames. A frame with extra food was provided during the experiment if food stores became insufficient. Enough bees were added to occupy at least 2–3 frames completely. No queen cells were present on the combs when the experiment started.

The first series of colonies was installed on 9 June (table I, colonies 1–10), the second series on 27 July (table I, colonies 11–20) and the third series on 5 August (table I, colonies 23–26). The colonies of the second series (11–20) were separated from colonies into which a queen of European origin (who probably mated mostly with Africanized males) had been introduced 2 years before. Natural queen replacement might have taken place since that time. The other 12 hives had never received substitute queens from the beekeeper and were presumed to be strongly Africanized. No morphometric analysis was performed to quantify the racial differences between the colonies.

Every 2 d, the colonies were inspected to record: 1) the number of queen cells and their stage of development; 2) the number of empty queen cups; and 3) the presence of worker-laid eggs. These inspections were carried out until young queens emerged. After this, the hives were inspected less frequently to determine when the young queen started to reproduce.

During the first inspection, a sample of 50 of the workers was taken for ovary analysis. A second sample was taken at the estimated day of emergence of a new queen. The level of ovary activation was determined after dissection according to Velthuis (1970). He classified the ovaries into 3 stages, which can roughly be characterised as: 1) no eggs present in the ovarioles; 2) small eggs (surrounded by a closed epithelium) present; and 3) full-grown eggs present in the ovarioles. The number of ovarioles per single ovary was also counted, although this was not done systematically in the first series of 8 colonies. Since it is difficult to determine the number of

ovarioles when the ovaries are completely inactivated (Velthuis, 1970), this was only done for workers with at least a low level of ovary activation (consequently, $n < 50$ in all cases).

RESULTS

Table I summarises the results of this study. There was no significant difference between colonies 11–20 (produced from colonies into which a European queen, mated with Africanized drones, had been introduced 2 years earlier) and the other colonies, with respect to ovary activation and the number of queens raised (Mann–Whitney U test, $P > 0.05$). Significantly more empty queen cups were constructed by colonies 1–10 than by colonies 11–20 (Mann–Whitney U test, $Z_{corr} = -3.093$, $P < 0.01$). Because no important differences were found, no distinction will be made between the colonies of the different series in the rest of the analyses presented here.

Egg-laying workers

Two of the colonies contained many worker-laid eggs within a week after becoming queenless (table I). In hive 1, the workers laid thousands of eggs in most of the cells of 2 combs. A new queen emerged after 11–12 d. After this, we no longer found any new worker-produced brood. The queen started to lay eggs another 12 d later. Some of the worker-produced brood reached adulthood, but most did not (this was not quantified). Many eggs (> 1 000) were laid in 3 combs by workers of hive 4. Two queens were raised in this hive but both disappeared (for unknown reasons), so the hive remained queenless and workers continued to lay eggs. Again, only a few drones from these eggs reached adulthood. In 7 colonies, it was observed that egg laying took place on a very small scale (1–10 eggs found). Most of these eggs were found in extremely large cells, such as empty queen cups. All of these eggs disappeared before, or shortly after, hatching. No more worker-laid eggs were found in any of the colonies once a virgin queen had emerged.

Ovarian activation

No workers with activated ovaries (stage 2 or 3, Velthuis, 1970) were found in the samples of workers that were taken after 2 d of queenlessness.

The ovarian activation at the time of emergence of the first young queen, *ie* after ± 11 d, was very variable (table I). Workers with activated ovaries were found in every colony; workers with mature eggs (stage 3) were found in 9 colonies.

Remarkably, no workers with ripe eggs in their ovaries were found in the sample of hive 4, in which a large number of worker-laid eggs was found. This may indicate that reproduction in this hive was restricted to a relatively small number of workers which were not well represented in the sample.

Number of ovarioles

The average number of ovarioles per bee was very variable between colonies (table I). Because of the large standard deviation within the groups, the pair-wise differences between colonies were significant in only a few cases (table I, the ANOVA F-test showed a highly significant differences between the means: $DF = 392$, $F = 8.314$, $P < 0.0001$; the results of pair-wise comparisons by means of the Scheffé-F test are indicated). The maximum numbers of ovarioles per ovary ranged from 9 to 17. In workers from a queenright swarm, which was not part of the experiment, a maximum number of 19 ovarioles per ovary was found

Table I.

Colonies [a]	*Queen cells*	*Empty cups*	*% of worker ovaries* [b] *Stage 2*	*Stage 3*	*Worker eggs observed*	*Time to first worker eggs (d)*	n [d]	*No of ovariole* [c] *Average* [e]	*Max*
1	12	2	24	10	> 1 000	4		?	10
2[f]	5	4	6	4	–			?	
4	2	8	10	0	> 1 000	6		?	17[g]
5	6	21	20	0	1–10	8	12	8.33 [A]	11
6	7	5	10	0	–			?	
7	9	13	18	4	1–10	8	7	6.86 [A,B,C,D]	12
8	4	5	12	2	1	6		?	
10	11	7	16	0	–		18	7.61 [A,B]	16
11	4	1	8	0	–		24	4.92 [A,B,C,D]	11
12	9	1	6	0	1	8	29	7.76 [A]	12
13	4	1	28	2	–		30	6.23 [A,B,C,D]	12
14	5	0	2	0	–		24	5.75 [A,B,C,D]	12
16	8	3	16	4	1–10	6	42	3.79 [D]	9
17[h]	5	1	12	0	–		27	4.44 [B,C,D]	10
18[i]	10	4	16	0	–		23	4.91 [A,B,C,D]	10
20	8	1	14	0	–		25	5.36 [A,B,C,D]	12
23	5	3	40	2	1–10	6	35	5.60 [A,B,C,D]	13
24	11	1	12	4	–		32	6.66 [A,B,C]	12
25	7	10	62	4	–		37	4.27 [C,D]	9
26	5	7	10	0	1–10	10	28	5.39 [A,B,C,D]	9
S1			0	0			15	7.33 [A,B,C,D]	19
S2			0	0			15	7.40 [A,B,C,D]	11

[a] The missing numbers represent colonies which absconded shortly after installation (4 x) or were destroyed by ants (genus *Eciton*, 2 x). [b] Samples of 50 workers taken at the estimated day of emergence of a new queen. [c] The number of ovarioles was not systematically investigated in the first series. [d] The number of ovarioles was only determined if some ovary activation had taken place, so $n < 50$ in all cases. [e] Averages which are followed by at least 1 similar letter are not significantly different (ANOVA, Scheffé-*F* test, $P > 0.05$). [f] This colony was taken from a hive in which we found 2 queens, apparently both producing eggs. [g] Observed in 2 workers. [h] At the day when the first emergency queen cell was supposed to open, a fertilised, egg-producing queen (with her spermatheca full of sperm) was found in the colony. [i] This colony became very large (8 frames completely covered with bees) after bees from a neighbouring colony flew in in a large numbers.

(data from 2 such swarms are included in table I). The averages ranged from 3.79 to 8.33.

No correlation was found between the average number of ovarioles and the average level of ovarian activation (number of workers with ovaries in stage 2 or 3) if the colonies were compared (Spearman rank correlation test: over all colonies; R_{corr} = −0.146; $P > 0.05$). Moreover, within the colonies, no correlations were found between the number of ovarioles and the level of ovarian activation in individual workers (rank correlation tests over all workers, NS in all colonies).

Number of queen cells

The average number of queens that was raised (table I) was 6.85 per colony, although there was a large variation (minimum 2, maximum 12). The number of queens raised was not correlated with the percentage of workers with ovarian activation (stages 2 + 3) (Spearman rank correlation test: $N = 20$; $R_{corr} = 0.323$; $P > 0.05$).

Number of empty queen cups

Empty queen cups were constructed in 19 out of 20 colonies, only in the first days of queenlessness. After the second inspection (*ie* after 4 d), no newly constructed empty cups were ever found. The occurrence of empty queen cups did not correlate with the number of queens raised (Spearman rank correlation test: $N = 20$; $R_{corr} = 0.022$; $P > 0.05$), or with the number of workers with activated ovaries ($N = 20$; $R_{corr} = 0.326$; $P > 0.05$). Queens were never raised in cells that were originally found to be constructed as empty cups. This indicates that transportation of diploid brood into these cells did not occur (although it cannot be excluded that brood was transported into an empty cell during the first 2 d after dequeening). As mentioned above, empty cups were favourable places for worker ovipositions.

DISCUSSION

As previously reported by van der Blom and Arce (1991), laying workers of Africanized bees developed quickly, in spite of the presence of young brood and queen cells. Contrary to the findings of Jay (1969, 1972) and Hepburn *et al* (1988, concerning *A m capensis*), the brood apparently did not have a strong inhibiting effect on ovary activation in workers in these colonies.

A large variation was found among the colonies with respect to the development of laying workers. This may be explained partly by the relatively recent 'mixing' of European races with the Africanized bees. The number of ovarioles resembled the values of *A m ligustica* in some cases (averages of 3.7 ovarioles per ovary are found by Weaver[1], 1956, and Ruttner and Hesse, 1981) and *A m scutellata* in other cases (Ruttner and Hesse (1981) found an average of 7.1 for this race, whereas Hepburn and Crewe (1991) found values for *A m scutellata* which are much lower, indicating that there is a large local variation with respect to this trait). Differences in ovarian activation were not correlated with the numbers of ovarioles at the individual level (this was also found by Velthuis, 1970). The number of ovarioles and the speed of ovarian activation therefore seem to be independent characters in a

[1] This number was found by taking the average of all the numbers of ovarioles from all the workers in all the experiments and dividing by 2, since Weaver gave number of ovarioles per bee, and not per ovary as later authors did.

mixed population. In our present study, some colonies had a low average number of ovarioles per worker (as *A m ligustica*), but a quick ovarian activation (as *A m scutellata*), whereas the other combinations also occur.

No more worker-laid eggs were found after a young queen had emerged, although in most colonies there was a considerable number of workers with fully activated ovaries at that time. This illustrates the importance of the presence of a queen, who did not start reproducing herself until 7–14 d after emergence. The same effect of virgin queens was found in *A florea* (Woyke and Wongsiri, 1992) and *A cerana* (Boot *et al,* unpublished data).

Since no correlation was found between the number of queens that was raised and ovarian activation, there are no indications that the occurrence of laying workers interferes with the raising of new queens. This might have been expected since van der Blom (1991) found that future laying workers were involved in queen rearing slightly more than workers without ovary activation. The experience of bee keepers is that Africanized colonies are excellent nursing colonies for queen rearing.

Laying workers under broodright conditions have been reported to occur in *A m adansonii* (Fletcher and Ross, 1985), *A m yemenitica* (Woyke, 1993), *A cerana indica* (Millen, 1942; Ribbands, 1953; Boot *et al,* unpublished data) and *A florea* (Woyke and Wongsiri, 1992). Ruttner and Hesse (1981) found a rapid ovarian activation in 3 African races: *A m scutellata*, *A m intermissa* and *A m capensis*. Furthermore, laying workers may occur frequently in *A dorsata* (Velthuis *et al,* 1971). Workers of certain tropical honeybee varieties thus seem especially able to lay eggs soon after becoming queenless, even under broodright conditions. Variation in this trait among races may be the result of a different balance between the positive and negative selection pressures with respect to the occurrence of laying workers. The positive selection pressure is reflected by the chance that the worker-produced drones mate successfully; the negative pressure depends on the relative costs of the production of worker-produced males. Climatic factors which determine the length of a yearly reproduction season, and the length of possible dearth periods, are very likely to influence these selection pressures with respect to the occurrence of laying workers.

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Résumé — Les ouvrières des colonies d'abeilles africanisées (*Apis mellifera* L) au Costa Rica élèvent des reines et pondent simultanément. Vingt petites colonies orphelines d'origine mixte européenne et africaine ont été pourvues en jeune couvain et en nourriture abondante. On a observé tous les 2 j le développement de cellules royales de sauveté et la présence éventuelle d'œufs pondus par des ouvrières. Des échantillons d'ouvrières ont été prélevés pour examiner le niveau du développement ovarien. i) On n'a trouvé aucune ouvrière ayant des ovaires développés après 2 j d'orphelinage. Dans 2 colonies, les ouvrières pondeuses étaient présentes en grand nombre au bout de 5 à 7 j (*ie* avant que les reines n'émergent des cellules de sauveté). Un petit nombre d'œufs pondus par des ouvrières a été trouvé dans 7 colonies. Dans les autres le niveau de développement ovarien des ouvrières était très variable. ii) On n'a trouvé aucun œuf pondu par des

ouvrières dans aucune colonie après qu'une reine ait émergé, même s'il fallait attendre jusqu'à 2 sem pour que la jeune reine se mette à pondre. iii) On n'a trouvé aucune corrélation entre le niveau du développement ovarien et soit le nombre de reines élevées dans des cellules, soit le nombre de cupules de reines vides construites. iv) Le nombre moyen d'ovarioles par ouvrière a beaucoup varié d'une colonie à l'autre (entre 8,33 et 3,79 par ovaire). v) On n'a pas trouvé de corrélation significative entre le nombre moyen d'ovarioles et le niveau moyen du développement ovarien d'une colonie à l'autre, ni entre le niveau du développement ovarien et le nombre d'ovarioles chez les ouvrières au sein d'une même colonie. Nous en concluons que la vitesse de développement des œufs dans les ovaires et le nombre d'ovarioles sont des caractères indépendants chez les populations mélangées. La pression de sélection positive concernant les ouvrières pondeuses dans une colonie avec couvain dépend de la probabilité pour que les mâles issus d'ouvrières puissent réussir à s'accoupler. La pression négative est déterminée par les coûts relatifs de la production de mâles issus d'ouvrières. Il est tout à fait possible que l'équilibre entre ces facteurs soit lié aux conditions climatiques qui règnent dans les habitats d'origine des diverses races d'abeilles.

abeille africanisée / biologie population / ouvrière pondeuse / ovaire

Zusammenfassung — Gleichzeitigkeit von Weiselaufzucht und Eilage von Arbeiterinnen in Bienenvölkern afrikanisch-europäischer Herkunft in Costa Rica. In zwanzig kleinen weisellosen Völkern mit Honigbienen europäisch-afrikanischer Herkunft, die junge Bienenbrut und reichlich Futter enthielten, wurde alle 2 Tage die Entwicklung von Nachschaffungszellen und die Anwesenheit von Arbeiterinneneiern registriert. Von den Arbeiterinnen wurden Proben genommen und der Entwicklungszustand ihrer Ovariolen untersucht. Nach 2 Tagen Weisellosigkeit wurde keine Arbeiterin mit aktivierten Ovariolen gefunden. Nach 5–7 Tagen enthielten zwei der Völker eierlegende Arbeiterinnen in grosser Anzahl, also bereits bevor die Königinnen aus den Nachschaffungszellen geschlüpft waren. In sieben der Völker wurden geringe Anzahlen von Arbeiterinneneiern gefunden. In den anderen Völkern waren die Arbeiterinnenovariolen in sehr unterschiedlichem Ausmaß aktiviert. Nachdem eine Königin geschlüpft war, wurden in keinem der Völker Arbeiterinneneier gefunden, obwohl bis zu 2 Wochen vergehen konnten, bevor die Königinnen in Eilage gingen. Zwischen dem Aktivierungsgrad der Ovariolen und der Anzahl aufgezogener Königinnenzellen bzw der Anzahl leerer Weiselnäpfchen bestand kein Zusammenhang. Zwischen den Völkern bestanden große Unterschiede in der mittleren Anzahl von Ovariolen pro Arbeiterin (zwischen 8,33 und 3,79 pro Ovar). Die Anzahl von Arbeiterinnenovariolen und der Aktivierungsgrad korrellierten weder in Bezug auf die Mittelwerte der Völker noch in Bezug auf die einzelnen Arbeiterinnen innerhalb der Völker. Hieraus wurde geschlossen, daß die Geschwindigkeit der Eientwicklung in den Ovarien und die Anzahl von Ovariolen unabhängige Eigenschaften dieser gemischten Population sind. Der positive Auslesewert der Eilage bei Arbeiterinnen unter brutrichtigen Bedingungen hängt von der Wahrscheinlichkeit ab, daß sich die arbeiterinnenerzeugten Drohnen erfolgreich verpaaren können, während der negative Auslesewert von den Kosten der Erzeugung dieser Drohnen bestimmt wird. Möglicherweise wird das Gleichgewicht zwischen diesen Faktoren von unterschiedlichen klimatischen Bedingungen in den Herkunftshabitaten der Honigbienenvarietäten beeinflusst.

afrikanisierte Honigbiene / Populationsbiologie / eierlegende Arbeiterin / Eierstock

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