CHALEPOXENUS BRUNNEUS, A WORKERLESS "DEGENERATE SLAVE-MAKER" ANT (HYMENOPTERA, FORMICIDAE)

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Chalepoxenus is a genus of parasitic ants with several closely related species mainly occurring around the Mediterranean (Buschinger et al., 1988). *C. kutteri* and *C. muellerianus* as well as *C. insubricus* and *C. siciliensis* (the latter two presumably synonymous to *C.m.*) are actively dulotic species having a considerable worker-force and enslaving several *Leptothorax* species (Ehrhardt 1982, 1987). Their sexuals engage in essentially normal mating flights, and new colonies are founded through single queens who penetrate a host nest, kill all adults by stinging them, or drive them off, and take over the broods from which the first slaves emerge.

Little has been known on the life history of *C. brunneus,* however. The species was described (Cagniant 1985) after a number of males and females which emerged in laboratory culture from a colony of *Leptothorax* cf. *marocanus* Santschi. This colony had been collected near the pass road to Tizi-n'-Test, 2000 m, in the Great Atlas of Morocco.

During a second visit to the type locality by A.B., H.C., J.H. and X. Espadaler on 6 May 1987, a total of 11 colonies with queens and/or sexual pupae of *C. brunneus* was collected. Subsequent laboratory observations revealed that this species is in fact worker-less, and that the young queens are accepted by most of the adult host colony workers. Apparently *C. brunneus* has reached the stage of a "degenerate slavemaker", convergently to some species of the genus *Epimyrma* (Buschinger, in press).

MATERIALS AND METHODS

The colonies of *C. brunneus* and its host species were found beneath flat rocks in the soil and debris. The fairly complete societies were aspirated into vials, and carried back to Germany in PVC

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tube-nests as described by Buschinger and Heinze (in press). In the lab, the colonies were kept in formicaries according to Buschinger (1974), and fed three times a week with diluted honey and insect pieces (*Tenebrio molitor* pupae, cockroaches). Artificial daily and annual temperature cycles were applied in analogy to that which had proved to be effective in rearing other leptothoracines from high elevations in the Alps.

Thus, "summer" conditions with 10h/17°C and 14h/28°C daily (dim light during the warm hours) were provided from the arrival in the lab on 28 May 1987 for 6 weeks, until most sexuals had left the nests for mating, and pupation had ceased. "Fall" conditions were simulated with 12h/15°C and 12h/25°C for another 6 weeks, followed by 12h/10°C and 12h/20°C for 3 weeks until the larger larvae had somewhat shrivelled. The colonies then were placed in hibernation conditions at a constant 10°C for five months. After 2 weeks at 10/20°C and 6 weeks at 15/25°C the first new prepupae appeared in the nests, and in 17/28°C a week later sexual pupae were observed. The whole "annual cycle" thus was shortened to about 10 months. No different temperature regimes were tested, but might as well be suitable.

Sexual behavior was observed either spontaneously in the formicaries, or sexuals who had left the nest chambers and were crawling and fluttering around were placed into a transparent flight-cage (15 × 20 × 30 cm) exposed to natural or artificial light. Females were dissected for control of insemination as was described by Buschinger and Alloway (1978). For colony foundation experiments single newly inseminated females, or dealate ones found in the formicaries, were placed into a formicary containing a queenright or queenless host-species colony.

RESULTS

Sexual behavior:

As in most leptothoracine ants the young sexuals of *C. brunneus,* when ready to mate, leave the nest and, in our formicaries, crawl or flutter around. When transferred into the flight-cage, they move towards the light and assemble in a loose "swarm" like sexuals of other *Chalepoxenus* species. The *C.b.* sexuals, however, are generally quite sluggish with respect to flying.

Mating was directly observed four times in a flight-cage, on 11, 12 and 15 June, 1987, hence about 2 weeks after the sexuals had hatched. The colonies were kept in a daily temperature rhythm of 17/28°C with the temperature increase at 3 a.m. Sexual behavior was observed at 3 to 6 p.m., thus about 12 to 15 hours after the morning rise in temperature.

The females did not exhibit any conspicuous behavior indicating a sexual calling or other stimulating actions. Males often made mating attempts already in the chambers of the formicaries, and one copulation was also seen there. Dealate females found in the formicaries often proved inseminated when dissected. In one colony 11 out of 12 dealate females were inseminated, only one had an empty receptacle. Such females, thus, could be used for colony foundation experiments.

A sexual pheromone is present in the poison glands of *C. muellerianus* and *C. kutteri.* Pieces of filter paper soaked with the content of such a gland attract males and stimulate homosexual mounting attempts. The secretion is interspecifically active, and even cross-breeding of *C.m.* and *C.k.* was possible (Ehrhardt 1987). In order to check for a sexual pheromone in the poison glands of *C. brunneus* a few preliminary experiments were made. However, their number was restricted due to the small number of sexuals available, and the somewhat unexpected results certainly would deserve confirmation with more material.

In repeated experiments the *C. brunneus* males failed to show any reaction to the poison gland content of their own females, and *C. muellerianus* males also were not stimulated by *C.b.* glandular secretion. Immediately after the test the same *C.m.* males showed the usual reaction to the sexual pheromone of conspecific females. When *C. muellerianus* poison gland secretion was presented to *C. brunneus* males, however, they were also attracted and stimulated and eventually engaged in homosexual contacts. Thus, *C. brunneus* has perhaps lost the ability to produce (much of) a sexual pheromone, but the males have retained the capability to perceive the (similar or identical) pheromone of congeneric females.

Colony foundation:

When collected in the field, six out of 12 colonies (including the first one of Cagniant, 1985) contained one *C. brunneus* queen each,

in one colony we found two queens, and five colonies were queen-less. The latter were orphaned already in the field, or we had lost the queens during collecting. None of the 12 colonies contained a host-species queen, but most had a *C. brunneus* brood, the small pupae being easily identifiable. A variable number of about 15 to 80 *L. marocanus* workers was present.

These field records suggest that the host species queens might be eliminated by the *C. brunneus* queens, as in the related dulotic *Chalepoxenus* species. With a total of 24 dealate *C.b.* females, colony foundation experiments were set up, using 6 queenright and 4 queenless *L. marocanus* colonies (Tab. I). Since many *C.b.* females died soon or were killed by the host-colony workers, up to four *C.b.* females were introduced subsequently into one nest. In a few instances (col. no. 5 and 8) where the *C.b.* females died during or after their first hibernation, newly reared females were introduced in the following "summer". Out of 20 females which died during the experiments, nine could be dissected; 6 of them were inseminated and 3 were not. Thus, lack of insemination cannot explain the failure of so many females.

In the four queenless host-colonies a total of 9 *C.b.* females were introduced. Only one (col. no. 1) was successful in that male and female offspring were reared in the year after the colony foundation. In another colony (no. 4) only one *C.b.* male was produced.

Fifteen *C.b.* females were placed into the six queenright host colonies. In two colonies both male and female offspring were reared; in two others only one and seven males, respectively, were reared.

Most interesting is the behavior of the *C.b.* females toward the host colony queens. Unfortunately, the results are somewhat ambiguous. Clearly, in colonies no. 6 and 9, the *C.b.* females killed the host colony queens. In col. 6 this happened 27 days after the *C.b.* female had been introduced into the nest. The *L.m.* queen was presumably stung; she was paralyzed, lying on her side or back, and a week later she was dead and carried out of the nest. Nevertheless, the *C.b.* female in this nest did not reproduce. In col. 9 a total of three *C.b.* females were killed first by the host workers (two were not inseminated, the third one could not be dissected), until the fourth *C.b.* female finally succeeded in paralyzing the *L.m.* queen, 9 days after the introduction. During this time the *C.b.* female had been staying within the nest, among the workers and brood; she had

Tab. 1. Colony foundation experiments with dealate *Chalepoxenus brunneus* females and queenright or queenless *Leptothorax marocanus* colonies.

L. marocanus coll. no.	L.m. queen	C. brunneus queens introduced (dead after n days) (i = inseminated[1])	C.b. brood produced		Remarks
1	absent	1. (surviving)[2] 2. (35,i) 3. (17,i?)	9♂	15♀	2 L.m. ♀♀ also produced
2	"	1. (33,i) 2. (37,i) 3. (3,i?)	—	—	L.m. ♂♂ produced
3	"	1. (1,i?) 2. (60,i)	—	—	♀♀ and ♀♀ L.m. produced
4	"	1. (13,i)	1♂		1♂ L.m. produced
5	present	1. (247,i?) 2. (7,i?)	7♂		♀♀ and ♀♂ L.m. produced, C.b. ♀♀ coexisted with surviving L.m. queen
6	"	1. (254,i?)	—	—	♀♀ and ♀♀ L.m. produced, L.m. ♀ killed
7	"	1. (22,i?) 2. (surviving)	5♂	10♀	1st C.b. ♀ paralyzed L.m. ♀ which recovered, 2nd C.b. ♀ coexists with surviving[2] L.m. queen. ♀♀ and ♀♂ L.m. produced.
8	"	1. (10,i) 2.(235,i?) 3. (surviving)	1♂		♀♀ and ♀♀ L.m. produced, L.m. ♀ surviving, coexistence
9	"	1. (1,ni) 2. (1,i?) 3. (2,ni) 4. (surviving)	3♂	15♀	4th C.b. ♀ killed L.m. ♀; L.m. ♀♀ ♂♂ produced together with C.b. sexuals
10	"	1. (13,ni) 2. (26,i?) 3. (28,i?)	—	—	many ♀♀ and ♀♂ L.m. produced L.m. ♀ surviving

1) i = inseminated; ni = not inseminated; i? = organs too decayed to permit determination whether or not inseminated.
2) surviving until second hibernation (August 1988).

paralyzed at least 12 *L.m.* workers (see below), and her gaster became visibly swollen. The *L.m.* female remained paralyzed, in the nest, for 40 days until she had died. This colony produced a considerable number of *C.b.* offspring (3 males, 15 females), but also some *L.m.* females and workers after the hibernation.

In colony no. 7, the first *C.b.* female introduced was seriously attacked by the host workers. She paralyzed 18 out of the 30 workers present, and on day 7 the *L.m.* female also was paralyzed. Five days later the host queen had recovered, however, and another 10 days later the *C.b.* female was dead. A second *C.b.* female was introduced, and after short aggressions through the *L.m.* workers she was accepted and survived for more than a year. Both the host and the parasite queen coexisted, and the colony produced 5 males and 10 females of *C. brunneus*.

Colony no. 5 and no. 8 represent two other instances where coexistence of the host and parasite queens occurred for a long time. In col. 5 the first *C.b.* female, after a short aggression, was accepted by the host workers, stung and paralyzed a total of 7 among them, and remained in the nest together with the host queen until she died 247 days later, after the hibernation. A second *C.b.* female, introduced in the next "summer" period, survived less than a week. The colony produced *L.m.* offspring and 7 *C.b.* males. Col. 8 had a similar fate, with the second *C.b.* female coexisting with the *L.m.* queen for 235 days, and a third *C.b.* female living in the colony for 2 months. The production of this colony, however, was only 1 *C.b.* male and a number of host workers and females.

As was mentioned above, the *C.b.* females, having penetrated the host nest, are usually attacked by the host workers, and sometimes quickly killed through biting and pulling off their appendages. When the *C.b.* female escapes these attacks, and sometimes very soon (within one hour after first having entered the nest), she stings several host workers, which are then paralyzed, lying inside or outside the nest for as long as a week, with slightly trembling appendages. Most of them eventually die; only a few may recover. Paralyzation of host workers also occurred in orphaned *L.m.* colonies; thus in col. no. 1 seven out of 19 workers were stung to death, and in col. no. 3, nineteen out of 30. The stinging of host workers may continue for more than 2 weeks.

Tab. 2. Production of five *Chalepoxenus brunneus* colonies collected in the field (1st brood May/June, 1987; 2nd brood in April, 1988) and three founded in the laboratory (1st brood in April, 1988).

	Production of sexuals					
	1st brood		2nd brood		total	
Field col. no.	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
1	15	56	5	8	20	64
2	4	8	2	2	6	10
3	1	1	4	4	5	5
4	=	=	6	16	6	16
5	3	9	8	6	11	15
Sex ratio ♂/♀ = 0.44					48	110

	1st brood					
Lab col. no.	♂♂	♀♀				
1	9	15				
7	5	10				
9	3	15				
	17	40		♂/♀ = 0.42		
All colonies	65	150		♂/♀ = 0.43		

Brood production and sex ratio

In laboratory-founded colonies the *C.b.* females usually develop a somewhat swollen gaster even during the second week after having penetrated a host colony. Egg-laying apparently begins soon after, since higher egg numbers were recorded about 2–3 weeks after the colony founding, when the *C.b.* female survived that long. Egg-laying continues until the fall; the female of col. no. 1 was seen to lay an egg on day 73 after colony founding.

Evidently there is no "rapid brood" production since in all colonies the first *C.b.* sexual pupae appeared in the "summer" after colony founding and hibernation, having developed from hibernated larvae.

No *C.b.* workers were ever produced, neither in the field-collected colonies nor in the laboratory-founded ones. Only in one field colony a worker pupa with ocelli, perhaps an intermorph, was recorded; however, a week later it was destroyed.

The number of *C.b.* sexuals produced was highly variable, certainly in part dependent upon the numbers of host workers in the

colonies. Tab. II reveals the records from field and laboratory colonies. The sex ratio (male/female) is close to 0.43 both in the field and laboratory colonies, thus markedly female-biased. This is not substantially changed when the three laboratory-founded colonies, which only produced males (Tab. I, no. 4, 5, 8), are included; the sex ratio then would be 0.49, still more than 2 females per male.

DISCUSSION

Our field and laboratory data clearly reveal that *Chalepoxenus brunneus* is a workerless parasitic ant, in contrast to the other species of this genus. Except for *C. spinosus,* of which only alate sexuals are known (Buschinger 1987), all other species have a worker caste, and *C. muellerianus* (including *C. insubricus* and *C. siciliensis*) as well as *C. kutteri* have been shown to conduct slave raids. For *C. tramieri* (Cagniant 1983) dulotic life habits are also assumed. *C. brunneus* thus represents a "degenerate slavemaker" like several species in the genus *Epimyrma,* which are parasites of *Leptothorax* species (s.g. *Myrafant* and *Temnothorax*) as is *Chalepoxenus* (Buschinger and Winter 1985, Buschinger et al. 1987, Buschinger in press).

As in the workerless *Epimyrma corsica* and *E. adlerzi,* the *C. brunneus* females apparently are able to eliminate the host colony queens during parasitic colony founding, using the genus-specific technique of stinging them, while *Epimyrma* females throttle the host queens to death. Different from the dulotic *Chalepoxenus* species, however, where the host queen soon dies when stung, the *L. marocanus* queens are only paralyzed by the *C.b.* female, and may even recover, as was shown in one experiment, or die only after several days. Another difference refers to the behavior towards the host colony workers: In *C. muellerianus* and *C. kutteri* the parasitic queen stings most of the workers to death (some workers and the queen often escape), and takes over only their broods. *C. brunneus,* on the other hand, stings only part of the host workers, which also do not die immediately (only after several days), and she is accepted by the remainder of the adult host colony workers. This parallels our observations in *Epimyrma,* again, in that during colony foundation the *Epimyrma* queens also sting a small number of host workers (Buschinger and Winter 1985, Buschinger et al. 1987, Douwes et al. 1988), and are accepted by the others. Adoption of the

parasitic queen by adult host workers, however, occurs in all *Epimyrma* species, not only in the degenerate slave-makers. This acceptance in *Epimyrma* thus is a preadaptive feature already present in the slave-making species; it is certainly favorable for a degenerate slave-maker who, when workerless, cannot replenish its slave stock through raiding. In *Chalepoxenus brunneus,* on the contrary, the queens had to evolve *de novo* the capability of being accepted by adult host workers.

It remains an open question of whether or not *C. brunneus* is on the way to an even more specialized type of coexistence with the host queens, a true inquilinism. Some of our experiments seemingly support such an assumption. However, the observed instances of coexistence between the *C.b.* and the host queens might well be artifacts due to inappropriate laboratory conditions. In the field such a coexistence has not been observed. And also in *C. muellerianus* and *C. kutteri*, in a total of now close to 500 field colonies checked, a coexistence of host and parasite queens has never been found. In laboratory colony founding experiments, however, it has been recorded in a couple of instances, also in these species (Ehrhardt unpubl.).

As was demonstrated in our laboratory cultures of *C. brunneus,* the production of sexuals is markedly queen-biased, with a numerical sex-ratio close to 0.43 (male/female). This might indicate a certain degree of inbreeding, with sexuals mating close to the maternal nests; in fact we saw mating behavior within our narrow formicaries. The apparent absence of a female sexual pheromone would fit to this assumption. Queen bias is usually found in such parasitic (and also free-living) species, where inbreeding occurs, most evidently in *Epimyrma* species with mating inside their nests (Buschinger and Winter 1985, Douwes et al. 1988). For a discussion of this feature see Buschinger (in press).

The *C. brunneus* population of Tizi-n'-Test, according to our observations, is very small, covering an area of not more than 50 × 100 m, and is certainly isolated. The species has not been found in close vicinity nor in farther distant sites of similar elevation, exposition, etc., despite our spending several days in search of additional localities. With an estimated population of less then 100 nests at any time a high degree of inbreeding is inevitable, even if sexuals do not mate exclusively with those of the same, or closely neighboring, nests.

In conclusion, we may state that *C. brunneus* is a workerless species, derived from actively dulotic congeners. The queens have evolved the capability to coexist with adult host workers of the colonies, which they invade for colony foundation. The higher initial number of host workers makes slave-raiding less necessary. The *C.b.* queens have retained (always?) the feature of killing the host colony queens through stinging, as their dulotic congeners.

Due to overgrazing and deforestation of the Great Atlas, as in other parts of Morocco, suitable habitats of *C. brunneus* have been largely destroyed. The population we studied is certainly but a tiny relic of a once much more widespread species, and it appears close to extinction. We do not know whether or not such relics exist elsewhere. It remains also questionable how much of the peculiar features of *C. brunneus* is due to the secondarily very small population size and isolation, and what was characteristic of the species as a whole.

SUMMARY

Chalepoxenus brunneus, found in only one very small site in the Great Atlas of Morocco, represents a workerless parasitic species which is derived from actively dulotic congeners ("degenerate slave-maker"). The host species is *Leptothorax* cf. *marocanus.* The *C.b.* queen apparently eliminates the host colony queen by stinging her. She also stings to death a considerable part of the host colony workers, but, different from the dulotic congeners, the *C.b.* queen then is accepted by the remaining adult host workers. The sexual production is queen-biased (sex ratio 0.43 male/female); mating presumably takes place close to the nest. We found no evidence for a female sexual pheromone, but the *C.b.* males react on the pheromone from poison glands of other *Chalepoxenus* species. The life history of *C.b.* parallels that of other "degenerate slave-makers" in the genus *Epimyrma.*

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