Kin structure and queen execution in the Argentine ant *Linepithema humile*

M. Reuter[1], F. Balloux[1,2], L. Lehmann[1], L. Keller[1]

[1]Institute of Ecology, University of Lausanne, Bâtiment de Biologie, 1015 Lausanne, Switzerland

[2] Present address: Institute for Cell, Animal, and Population Biology, University of Edinburgh, King's Building, West Mains Road, Edinburgh EH9 3JT, Scotland, UK

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Correspondence: Max Reuter
Institute of Ecology, ZEA
University of Lausanne
Bâtiment de Biologie
1015 Lausanne
Switzerland

Phone: +41 21 692 41 82
Fax: +41 21 692 41 65
E-mail: Max.Reuter@ie-zea.unil.ch

Abstract

Every spring, workers of the Argentine Ant *Linepithema humile* kill a large proportion of queens within their nests. Although this behaviour inflicts a high energetic cost on the colonies, its biological significance has remained elusive so far. An earlier study showed that the probability of a queen being executed is not related to her weight, fecundity, or age. Here we test the hypothesis that workers eliminate queens to which they are less related, thereby increasing their inclusive fitness. We found no evidence for this hypothesis. Workers of a nest were not significantly less related to executed queens than to surviving ones. Moreover, a population genetic analysis revealed that workers were not genetically differentiated between nests. This means that workers of a given nest are equally related to any queen in the population and that there can be no increase in average worker-queen relatedness by selective elimination of queens. Finally, our genetic analyses also showed that, in contrast to workers, queens were significantly genetically differentiated between nests and that there was significant isolation by distance for queens.

Keywords: social Hymenoptera, kin selection, spite, unicoloniality, population structure

Introduction

Queen execution is probably the most puzzling feature in the biology of the Argentine ant *Linepithema humile* Mayr (formerly *Iridomyrmex humilis* Mayr). Every spring workers eliminate up to ninety percent of the queens (Keller et al., 1989). These executions inflict a significant cost on the colony, leading to a loss of about seven percent of the overall biomass produced per year (Keller et al., 1989). Queen killing has so far been reported from two introduced populations of this species (France: Keller et al. 1989; USA: Markin 1970). Nothing is known about its occurrence in native populations in Argentina and Brazil.

Despite the interest that queen execution has elicited among evolutionary biologists (Hamilton, 1972; Fletcher & Ross, 1985; Keller et al., 1989; Bourke & Franks, 1995) only one study has attempted to unravel its adaptive significance. Keller et al. (1989) investigated whether workers eliminate physiologically inferior queens in order to maintain a high colony productivity. No significant difference in weight, rate of egg-laying, or quantity of sperm stored was found among queens that survived and those that were executed (Keller et al., 1989). Furthermore, age does probably not play a role because most queens are less than one year old when executed (Keller et al., 1989).

In this paper, we present the empirical test of an ultimate explanation of the phenomenon that had been proposed by Keller et al. (1989). The hypothesis, hereafter referred to as "kin-selected queen execution hypothesis", states that queen execution is a spiteful behaviour (Hamilton, 1970) whereby workers of a nest collectively eliminate queens to which they are on average less related (Keller et al., 1989). By doing so, workers may increase their average relatedness to future sexual brood. This might be important because several factors tend to decrease relatedness among nestmates. First, the introduced populations in which queen killing has been observed are of the unicolonial type, meaning that workers as well as reproductives are freely exchanged between nests (Markin, 1970; Keller et al., 1989; Passera, 1994). The exchange of individuals breaks up family structure and lowers relatedness among nestmates. Moreover, colonies of the Argentine ant are known to spend the winter in shared nests which in spring split up again into separate nests

(Newell & Barber, 1913). The continuous joining and fissioning of nests contributes to the mixing of colonies and hence the decline of average relatedness among nestmates.

According to the kin-selected queen execution hypothesis, the queens eliminated should be those who are on average less related to the workers in the nest. We tested this prediction using field-collected colonies that were observed in the laboratory. We furthermore quantified the degree of genetic differentiation between the nests for both queens and workers to determine whether workers were on average more related to queens from their colony than other queens in the population as assumed by the kin-selected queen execution hypothesis.

Methods

Nest sampling and maintenance:

Nests of the Argentine ant were collected in March 1998 in Port Leucate on the Mediterranean coast of Southern France. Twenty-three nests dispersed over about one kilometre (Fig. 1) were located on a detailed map, excavated and transported to Lausanne. In the laboratory the nests were transferred to separate plastic containers and all soil was removed. We determined the number of queens in each nest after removing those who had apparently suffered injuries during transport. Nests were supplied with a humidified artificial nest and ad libitum food (see, e.g., Keller & Passera, 1993). Queen executions started some days after transfer to the laboratory. The nests were checked at least twice a day and corpses were immediately removed and stored at -20°C. The execution of queens ceased about three weeks after colonies were collected. At this point, the remaining queens and a sample of workers were killed and stored at -20°C.

Genetic analyses:

Out of the 23 nests, we chose ten with a relatively high initial number of queens and a relatively large proportion of queens having been executed for genetic analysis. The selected nests were dispersed over almost the whole stretch of the sampling transect (Fig. 1, squares). For each of the ten nests all queens and twenty randomly chosen workers (i.e., a total of 402 individuals) were genotyped. DNA was extracted using a standard phenol-chloroform protocol. We amplified five microsatellite loci specifically designed for the Argentine ant, *Lhum-11*, *Lhum-13*, *Lhum-19*, *Lhum-35*, and *Lhum-62* (Krieger & Keller, 1999), following the protocol given by these authors. Alleles were scored independently by at least two different persons.

Statistical analyses:

Relatedness calculations were performed using the program RELATEDNESS 5.0.5 (Goodnight Software, gsoft.smu.edu/GSoft.html) which computes the relatedness measure proposed by Queller & Goodnight (1989). The totality of the 402 individuals genotyped was taken as the reference population. For each nest, we calculated the workers' average relatedness to queens that survived and to those that were killed. The standard error (SE) of the differences between the pairs of relatedness coefficients were obtained using the jackknifing procedure implemented in RELATEDNESS 5.0.5. We jackknifed separately both over nests and loci. The significance of the difference between the workers' relatedness to surviving and eliminated queens was tested with a paired t-test. Genetic differentiation between nests (F_{ST}) was estimated with the program FSTAT (Goudet, 1995) version 2.8 (www.unil.ch/izea/softwares/fstat.html) on queens and workers separately. Isolation by distance was tested with Mantel tests (Manly, 1991), determining the correlation of the matrix of pairwise F_{ST} / (1 - F_{ST}) values and a matrix containing the natural logarithms of geographic distances between nests (Rousset, 1996).

Results

In the ten nests used for genetic analyses, the initial number of queens was 20.3 ± 2.1. Of these queens 4.2 ± 1.5 (21 ± 12%) were executed. The percentage of queens eliminated was considerably lower than that observed in the field. However, fewer queens seem to be killed by workers in the laboratory than in the wild (Keller et al., 1989). The average relatedness of workers to surviving queens was 0.009 ± 0.083 (mean ± SD) while the relatedness to those who were killed was -0.008 ± 0.142. The difference in the workers' relatedness to surviving and killed queens was very small ($R_{surviving} – R_{killed}$ = 0.021) and not significantly different from zero (jackknifing over nests: SE = 0.041, t_{10} = 0.51, n.s.; jackknifing over loci: SE = 0.084, t_5 = 0.25, n.s.).

The genetic structure between the ten nests included in the genetic analysis was weak but significant when estimated over queens (F_{ST} = 0.018, P < 0.0001). This was true for three out of the five loci analysed (Table 1). Furthermore, we observed significant isolation by distance in queens (r = 0.302, P = 0.03). In contrast to queens, workers were not significantly genetically differentiated between nests (F_{ST} = 0.004, n.s.) and showed no significant isolation by distance (r = 0.008, n.s.).

Discussion

Our study did not provide evidence for the kin-selected queen execution hypotheses. The workers of a nest were on average not significantly less related to the queens they executed than to those they spared. Moreover, our population genetic analysis revealed that workers were not genetically differentiated between nests. This implies that workers are on average equally related to any queen, be it from their own or another nest. Consequently, there is no opportunity for workers to increase overall relatedness to queens in their colony by selectively eliminating queens. We can therefore refute Keller et al.'s (1989) original hypothesis that workers collectively eliminate queen so as to increase their average relatedness to reproductives in the nest.

The dismissal of Keller et al.'s (1989) hypothesis does not generally preclude nepotism as the force driving queen execution. In contrast to the original hypothesis which assumed that workers of a nest act collectively in eliminating queens less related to the ensemble of workers, workers might individually assess their relatedness to queens and kill the less related ones. Such a behaviour would not result in a significant change in average queen-worker relatedness because in the absence of genetic structure among workers the queens eliminated by one worker would be those closely related to another worker and vice versa. Individual nepotistic queen execution would therefore not be detectable by our experimental approach. A test of this hypothesis requires relatedness values on individual executing workers and their victims, data which is very difficult to obtain because it requires continuous monitoring of the experimental colonies in order to sample executing workers.

Nepotistic queen execution as described above would not increase the workers' average relatedness to the sexuals they raise and thus not augment their inclusive fitness. Such apparently non-adaptive behaviour can nevertheless persist because in unicolonial populations (such as the one studied here) there is little or no selection on worker behaviour (Queller & Strassmann, 1998). The reason is that workers are virtually unrelated to the brood they raise and consequently the cost of worker behaviour does not affect the production of related sexuals (neither the workers' own reproduction since they are sterile). Thus, the inclusive fitness of workers is zero whatever their behaviour, and there is no potential for natural selection acting against queen execution. Furthermore, even if weak selection occurred, it would act as to maintain queen execution. Any non-executing mutant would have a selective disadvantage because it would not eliminate unrelated queens while being itself eliminated by workers of other genetic lineages.

Given the absence of selection on worker behaviour in introduced unicolonial populations of *L. humile*, the origin of queen execution would probably have to be sought in native populations. In South America multicolonial populations exist in which relatedness among nestmates is significantly positive (J. Pedersen, T. Giraud and L. Keller, unpublished) and stronger population differentiation might make the elimination of unfamiliar queens selectively advantageous. Queen execution may thus be a remnant phenomenon of a possibly adaptive behaviour in the native habitat.

Although our study has failed to give a conclusive answer concerning the ultimate causes of queen execution, our population genetic analysis has revealed an unexpected and interesting result in showing that queens are genetically differentiated among nests whereas workers are not. Previous genetic studies came to the conclusion that introduced populations were genetically homogenous and that genetic differentiation occurred only at a very large geographical scale (Pedersen et al., 1999; Krieger & Keller, 2000; Tsutsui et al., 2000). However, most of these studies had been accomplished using samples of workers only (Pedersen et al., 1999; Krieger & Keller, 2000; Tsutsui et al., 2000). The only study including queens (Kaufmann et al., 1992) found that queens are not significantly related within a nest, indicating the absence of genetic structure. However, this study was based on a relatively small sample (eight nests, 5.6 ± 4.4 queens per nest) and applied two allozyme systems with little variability. Thus, this study was unlikely to reveal significant relatedness if the relatedness values were low, as stated by the authors themselves (Kaufmann et al., 1992).

The most plausible explanation for the divergence in genetic structure between queens and workers lies in the difference in their mobility. Workers probably leave their nest more frequently than queens (e.g., to forage outside the nest) and they are thus more prone to end up in a foreign nest, given that there is little or no aggression toward non-nestmate individuals (Keller & Passera, 1993; Tsutsui et al., 2000). It is yet unclear whether a lack of aggression between workers from different colonies is due to a loss of diversity at recognition alleles following a bottleneck (Tsutsui et al., 2000) or whether it is due to unusual selective pressures occurring in the introduced range of this species' geographic distribution as has been demonstrated in the fire ant *S. invicta* (Ross & Keller, 1995).

In conclusion, our study provides no support for the kin-selected queen execution hypotheses. Future studies will have to investigate the phenomenon on a finer scale to finally unravel its significance. Also, it would be useful to determine whether queen execution also occurs in native populations. This would allow to verify whether queen execution is an ancestral behaviour or whether it has evolved following the introduction of this ant to new habitats.

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References

Bourke, A.F.G. & Franks, N.R., 1995. *Social evolution in ants*. Princeton University Press, Princeton.

Fletcher, D.J.C. & Ross, K.G. 1985. Regulation of reproduction in eusocial Hymenoptera. *Annu. Rev. Entomol.* 30: 319-343.

Goudet, J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. *J. Hered.* 86: 485-486.

Hamilton, W.D. 1970. Selfish and spiteful behaviour in an evolutionary model. *Nature* 228: 1218-1220.

Hamilton, W.D. 1972. Altruism and related phenomena, mainly in social insects. *Ann. Rev. Ecol. Syst.* 3: 193-232.

Kaufmann, B., Boomsma, J.J., Passera, L. & Petersen, K.N. 1992. Mating structure and relatedness in a french population of the unicolonial ant, *Iridomyrmex humilis* (Mayr). *Insectes Soc.* 39: 195-200.

Keller, L. & Passera, L. 1993. Incest avoidance, fluctuating asymmetry, and the consequences of inbreeding in *Iridomyrmex humilis*, an ant with multiple queen colonies. *Behav. Ecol. Sociobiol.* 33: 191-199.

Keller, L., Passera, L. & Suzzoni, J.P. 1989. Queen execution in the Argentine ant *Iridomyrmex humilis* (Mayr). *Physiol. Entomol.* 14: 157-163.

Krieger, M.J.B. & Keller, L. 1999. Low polymorphism at 19 microsatellite loci in a French population of Argentine ants (*Linepithema humile*). *Mol. Ecol.* 8: 1078-1080.

Krieger, M.J.B. & Keller, L. 2000. Mating frequency and genetic structure of the Argentine ant *Linepithema humile*. *Mol. Ecol.* 9: 119-126.

Manly, B.F.J., 1991. *Randomization and Monte Carlo Methods in Biology*. Chapman and Hall, New York.

Markin, G.P. 1970. The seasonal life cycle of the Argentine ant, *Iridomyrmex humilis* (Hymenoptera, Formicidae), in southern California. *Ann. Entomol. Soc. Am.* 63: 1238-1242.

Newell, W. & Barber, T.C. 1913. The Argentine ant. *U.S.D.A. Bureau Entomol. Bull.* 122: 1-98.

Passera, L. 1994. Characteristics of tramp species. In: *Exotic ants, biology, impact, and control of introduced species* (D.F. Williams ed), pp 23-43. Westview Press, Boulder.

Pedersen, J.S., Balloux, F., Reuter, M., Krieger, M.J.B. & Keller, L. 1999. Maintenance of altruistic behaviour in non-equilibrium populations: a study of unicolonial ants. In: *Proceedings of the Seventh Congress of the European Society for Evolutionary Biology* (A. Fontdevila ed), pp II-230. Autonomous University of Barcelona, Barcelona.

Queller, D.C. & Goodnight, K.F. 1989. Estimating relatedness using genetic markers. *Evolution* 242: 1155-1157.

Queller, D.C. & Strassmann, J.E. 1998. Kin selection and social insects. *Bioscience* 48: 165-175.

Ross, K.G. & Keller, L. 1995. Ecology and evolution of social organization - insights from fire ants and other highly eusocial insects. *Ann. Rev. Ecol. Syst.* 26: 631-656.

Rousset, F. 1996. Equilibrium values of measures of population subdivision for stepwise mutation processes. *Genetics* 142: 1357-1362.

Tsutsui, N.D., Suarez, A.V., Holway, D.A. & Case, T.J. 2000. Reduced genetic variation and the success of an invasive species. *Proc. Natl. Acad. Sci. USA* 97: 5948-5953.

Table 1: Estimates of genetic differentiation (F_{ST}) between the queens of the ten nests analysed.

Locus	**F_{ST}**	**SE**
Lhum-11	-0.008	0.009
Lhum-13	0.0	0.006
Lhum-19	0.017	0.015
Lhum-35	0.026	0.022
Lhum-62	0.055	0.028
Average	0.018	0.011

Figure Legends

Figure 1: Location of the sampled nests in Port Leucate, France. Nests included in the genetic analysis are represented by squares.

Figure 1