

26. D. H. Sandweiss *et al.*, *Science* **273**, 1531 (1996).
 27. D. T. Rodbell *et al.*, *Science* **283**, 516 (1999).
 28. M. S. McGlone, A. P. Kershaw, V. Markgraf, in *El Niño: Historical and Paleoclimatic Aspects of the Southern Oscillation*, H. F. Diaz, V. Markgraf, Eds. (Cambridge Univ. Press, Cambridge, 1992), pp. 435–462.
 29. E. R. Cook, B. M. Buckley, R. D. D'Arrigo, M. J. Peterson, *Clim. Dyn.* **16**, 79 (2000).
 30. P. Chang, L. Ji, H. Li, *Nature* **385**, 516 (1997).
 31. K. R. Briffa *et al.*, *Clim. Dyn.* **7**, 111 (1992).
 32. S. R. O'Brien *et al.*, *Science* **270**, 1962 (1995).
 33. D. A. Hodell, M. Brenner, J. H. Curtis, T. Guilderson, *Science* **292**, 1367 (2001).
 34. See supplemental figure at Science Online (www.sciencemag.org/cgi/content/full/293/5533/1304/DC1).
 35. M. Cane, A. C. Clement, *Geophys. Monogr.* **112**, 373 (1999).
 36. We thank D. Ariztegui, M. Cane, C. Charles, A. Clement, H. Cullen, W. Curry, D. Hodell, T. Joyce, L.

Keigwin, T. Stocker, H. Thierstein, D. Oppo, G. Philander, and two anonymous reviewers for discussions and helpful comments. We also thank M. Scherer for scanning electron microscopy (SEM) and x-ray results shown in Fig. 1 that arose from a class project at the University of Miami. Supported by the Deutsche Forschungsgemeinschaft and NSF.

9 February 2001; accepted 12 July 2001

Queen Control of Sex Ratio in Fire Ants

L. Passera,¹ S. Aron,² E. L. Vargo,³ L. Keller^{4*}

The haplodiploid sex-determination system of ants gives rise to conflict between queens and workers over colony sex ratios, and the female-biased allocation ratios seen in many species suggest that workers often prevail in this conflict. We exchanged queens between male- and female-specialist colonies of the fire ant *Solenopsis invicta*. These exchanges quickly reversed the sex-ratio biases of adopting colonies. The sex ratio of queen-laid eggs differed strongly between male- and female-specialist colonies. These findings suggest that queens can force workers to raise male sexuals by limiting the number of female brood and help to explain why sex investment ratios lie between the queen and worker equilibria in this and many other ant species.

Social insects provide some of the most striking examples of elaborate cooperative behavior, yet life within colonies also entails conflicts (1–5). In ants, the most strident conflict concerns sex ratio, with workers favoring a more female-biased sex investment ratio than queens (1). This conflict arises because of the hymenopteran haplodiploid system of sex determination, whereby unfertilized eggs develop into males and fertilized eggs into females (1). As a result, workers in colonies headed by a single once-mated queen are three times more related to sisters ($r = 0.75$) than to brothers ($r = 0.25$). Because of this asymmetry in relatedness, the population-wide sex allocation ratio should equilibrate at 3:1 (female:male) if workers control the colony's investment in reproductive offspring. By contrast, because queens are equally related to their daughters and sons, an equal investment in male and female reproductives is expected if the colony's allocation of resources is under the control of the queen.

Sex ratios have been studied in many ants, and, across species, population-wide sex allocation ratios lie between the queens' and

workers' equilibria (1–3, 6–8). Because they control brood rearing and food flow in the colony, workers can bias sex allocation, for example, by selectively eliminating males and/or preferentially feeding females. Workers have indeed been shown to manipulate colony sex ratios by selectively eliminating male brood in some ants (9–13). Studies of species in which variation in social organization leads to intercolonial differences in relatedness asymmetries also reveal that workers in some species bias sex ratios adaptively by favoring sex allocation in females in those colonies with relatedness asymmetries greater than the population average (e.g., those headed by one singly mated queen) and favoring the production of males in colonies with lower than average relatedness asymmetries (e.g., those with multiply mated queens or several related queens) (14, 15). The fact that population-wide sex allocation is less female-biased than the workers' equilibrium or close to the queen equilibrium in some species (16) suggests that workers do not exert complete control over sex allocation but that queens have some measure of control over sex-ratio allocation in some social Hymenoptera. The mechanisms by which queens may exert this control have remained elusive (2, 16–18).

Here we test the hypothesis that queens may bias sex ratios toward males by limiting the number of female eggs in the colony (16–21), using the fire ant *Solenopsis invicta*, one of the best studied social insects (22). Colonies of the monogyne form of this spe-

cies consist of one singly mated queen and her daughter workers that are completely sterile (23). This simple family structure leads to uniformly high relatedness asymmetry (3:1) and workers favoring an investment in females three times higher than queens. In this species, colonies produce mostly individuals of a single sex (24–26). To test whether sex-ratio specialization is induced by queens or workers, we made use of the fact that colonies made queenless will usually accept a new unfamiliar queen (27). The ability to conduct cross-fostering experiments, where queens are transferred between male- and female-specialist colonies, provides an opportunity to determine the extent to which queens or workers control colony sex ratios in a social insect.

We selected 24 field colonies with highly biased sex ratios in a monogyne population (28). Eleven of these colonies were male specialists (numerical proportion of males, range: 0.77 to 1.0), and 13 were female specialists (numerical proportion of males, range: 0.0 to 0.09). The queen in each colony was exchanged with the queen from another colony, producing sexuals of the other sex (experimental colonies) or sexuals of the same sex (control colonies). Twenty-two of the 24 colonies accepted the foreign queen, and 21 of these colonies produced a new batch of reproductives 5 weeks after queen exchange. Male-specialist colonies that were given a queen from a female-specialist colony switched to producing mostly or only females (Fig. 1; $t_3 = 16.14$, $P < 0.0001$, paired t test). Conversely, female-specialist colonies that received a queen from a male-specialist colony switched to producing males (Fig. 1; $t_4 = 8.94$, $P < 0.001$, paired t test). By contrast, no significant change in sex ratio occurred in control colonies in which male-specialist and female-specialist colonies were given a queen from the same colony type (male specialist, $t_3 = 0.67$, $P = 0.55$, paired t test; female specialist, $t_5 = 0.54$, $P = 0.61$, paired t test). The fact that colony sex ratios are predominantly influenced by queens is also demonstrated by the significant correlation between the sex ratio produced by a queen's original colony and the sex ratio produced by the recipient colony 5 to 6 weeks after the queen was introduced (experimental colonies: $r^2 = 0.77$, $n = 10$, $P < 0.001$; control colonies: $r^2 = 0.92$, $n =$

¹Laboratory of Ethology and Animal Cognition, FRE-CNRS 2382, University Paul-Sabatier, Toulouse Cedex 31062, France. ²Unit of Animal Communities, Free University of Brussels, Brussels 1050, Belgium. ³Department of Entomology, Campus Box 7613, North Carolina State University, Raleigh, NC 27695–7613, USA. ⁴Institute of Ecology, University of Lausanne, Lausanne 1015, Switzerland.

*To whom correspondence should be addressed. E-mail: Laurent.keller@ie-zea.unil.ch

REPORTS

10, $P < 0.0001$). In fact, there was no significant difference between the sex ratio of a queen's original colony and the sex ratio of the recipient colony after queen exchange (experimental colony, mean sex-ratio difference = -0.06 , paired t test: $t_{10} = 1.45$, $P > 0.17$; control colonies, mean sex-ratio difference = 0.02 , paired t test: $t_9 = 0.95$, $P > 0.3$).

The sudden shift in the proportion of males reared by experimental colonies after queen exchange demonstrates that colony sex

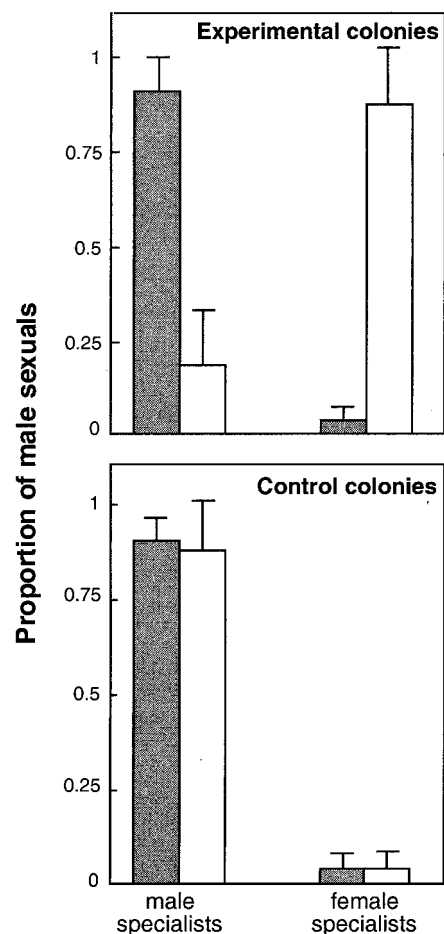


Fig. 1. Secondary sex ratios expressed as the numerical proportion of males (alates and pupae) in male-specialist and female-specialist colonies of *S. invicta*, before (black bars) and 5 to 6 weeks after (white bars) queen exchange experiments (mean \pm SD). (**Top**) In experimental colonies, queens were exchanged between male-specialist (number of colonies, $n = 6$) and female-specialist ($n = 5$) colonies; one female-specialist colony did not produce sexuals 6 weeks after queen exchange and was therefore removed from the analysis. (**Bottom**) In control colonies, queens were exchanged between colonies producing the same sex (male-specialist colonies: $n = 4$; female-specialist colonies: $n = 6$). Differences between colony sex ratios before and after queen exchange were compared with Student paired t tests on arcsine-transformed sex-ratio data. Reported values are back-transformed.

ratios are predominantly influenced by the queens, not the workers. To investigate whether the queen's influence is due to differences in the sex ratio of the eggs she produces (primary sex ratio), we determined the proportion of haploid (male) and diploid (female) eggs laid by queens from male- and female-specialist colonies using microsatellite markers (29). Primary sex ratio could unambiguously be determined in queens from 7 male- and 10 female-specialist colonies. There was a marked and significant difference between the two groups of queens ($t_{15} = 3.59$, $P < 0.003$). Queens from male-producing colonies laid more than 50% haploid eggs, whereas queens from female-producing colonies produced almost no haploid eggs (Fig. 2).

Our results show that queens have a strong effect over colony sex ratios by limiting the proportion of female eggs produced. Because male- and female-specialist colonies occur at equal frequencies in the population (26), workers have limited options to bias sex allocation. However, within these constraints, workers may still exert some measure of influence over sex investment ratios at the population level. A previous study showed that fire ant workers are able to recognize and selectively eliminate males (12). Workers eliminate male larvae in both male- and female-producing colonies, but males are al-

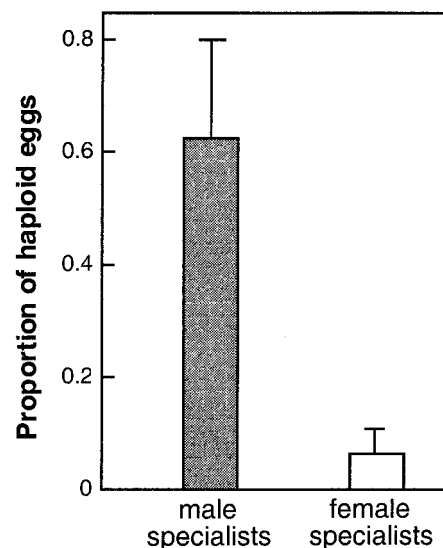


Fig. 2. Primary sex ratio expressed as the numerical proportion of haploid eggs laid by queens of male-specialist [number of queens, $n = 7$; mean number of eggs genotyped per queen: 34.3 (SD = 5.0)] and female-specialist [$n = 10$; mean number of eggs genotyped per queen: 28.7 (SD = 2.6)] colonies of *S. invicta*. Eggs were sampled before queen exchange experiments. The difference in the egg sex ratios between queens of the two groups was compared with an unpaired t test on arcsine-transformed sex-ratio data. Reported values are back-transformed.

most totally eliminated in the latter type. In fact, the sex investment ratio in monogynous populations of *S. invicta* lies between the queen and worker equilibria (1.5:1 in favor of females, proportional investment in females of 0.607) (26), suggesting that, at the population level, neither the queen nor the workers have full control over reproductive allocation in fire ants.

The finding that queens exert partial control over sex ratio in this species fits the prediction of a model investigating the outcome of queen-worker conflict over sex-ratio investment in situations in which queens control the primary sex ratio and workers control caste determination (i.e., whether female brood develops into workers or queens) and are able to recognize and eliminate males (21). This model, which assumes that male and female sexuals are produced simultaneously with workers, as indeed is the case in fire ants (30), predicts that queens will be selected to force workers to raise males by limiting the number of female eggs laid, whereas workers should respond to this limitation by raising a greater proportion of eggs as sexuals and thereby attain a more female-biased sex allocation. Consistent with the observed values of sex allocation ratios in populations of *S. invicta*, this tug-of-war between queens and workers is predicted to lead to a stable equilibrium where sex allocation is between the queen and worker equilibria and the investment in workers below both parties' equilibrium.

This report shows that, although workers in monogynous colonies of fire ants have the ability to eliminate males and bias sex investment ratio, the magnitude of their control is largely constrained by the primary sex ratio produced by queens. It is possible that queens in other ant species similarly exert some control over colony and population sex ratios by altering the primary sex ratio and limiting the number of female brood. The relative power wielded by queens and workers over sex allocation most likely will depend on various factors such as the degree of queen-male dimorphism and constraints on the ability of queens to limit the number of female brood (2, 19, 21). This may explain why sex-investment ratios frequently are less female-biased than predicted under worker control (2, 6, 7, 31) and why the departure from expected values varies greatly among species. More generally, this report shows that the outcome of queen-worker conflict over sex ratio may be more complex than generally assumed and that the relative power of queens and workers may vary according to life-history and ecological parameters.

References and Notes

1. R. L. Trivers, H. Hare, *Science* **191**, 249 (1976).
2. A. F. G. Bourke, N. R. Franks, *Social Evolution in Ants* (Princeton Univ. Press, Princeton, NJ, 1995).

3. R. H. Crozier, P. Pamilo, *Evolution of Social Insect Colonies. Sex Allocation and Kin Selection* (Oxford Univ. Press, Oxford, 1996).
4. L. Keller, H. K. Reeve, in *Levels of Selection in Evolution*, L. Keller, Ed. (Princeton Univ. Press, Princeton, NJ, 1999), pp. 153–175.
5. L. Keller, M. Chapuisat, *Bioscience* **49**, 899 (1999).
6. P. Nonacs, *Q. Rev. Biol.* **61**, 1 (1986).
7. J. J. Boomsma, *Am. Nat.* **133**, 517 (1989).
8. P. Pamilo, *Behav. Ecol. Sociobiol.* **27**, 31 (1990).
9. S. Aron, L. Passera, L. Keller, *J. Evol. Biol.* **7**, 403 (1994).
10. L. Keller, S. Aron, L. Passera, *Behav. Ecol.* **7**, 292 (1996).
11. M. Chapuisat, L. Sundström, L. Keller, *Proc. R. Soc. London Ser. B* **264**, 1255 (1997).
12. S. Aron, E. L. Vargo, L. Passera, *Anim. Behav.* **49**, 749 (1995).
13. L. Sundström, M. Chapuisat, L. Keller, *Science* **274**, 993 (1996).
14. D. C. Queller, J. E. Strassmann, *Bioscience* **48**, 165 (1998).
15. M. Chapuisat, L. Keller, *Heredity* **82**, 473 (1999).
16. K. R. Helms, *Evolution* **53**, 1470 (1999).
17. P. Pamilo, *Am. Nat.* **119**, 638 (1982).
18. J. Seger, *Science* **274**, 941 (1996).
19. M. G. Bulmer, P. D. Taylor, *Heredity* **47**, 197 (1981).
20. F. L. W. Ratnieks, H. K. Reeve, *J. Theor. Biol.* **158**, 33 (1992).
21. M. Reuter, L. Keller, *Am. Nat.* **158**, 166 (2001).
22. K. G. Ross, L. Keller, *Annu. Rev. Ecol. Syst.* **26**, 631 (1995).
23. D. J. C. Fletcher, K. G. Ross, *Annu. Rev. Entomol.* **30**, 319 (1985).
24. G. P. Markin, J. H. Dillier, *Ann. Entomol. Soc.* **64**, 562 (1971).
25. E. L. Vargo, D. J. C. Fletcher, *Physiol. Entomol.* **12**, 109 (1987).
26. E. L. Vargo, *J. Evol. Biol.* **9**, 783 (1996).
27. D. J. C. Fletcher, M. S. Blum, *Science* **219**, 312 (1983).
28. Thirty-five monogyne colonies of the fire ant were collected between 28 October and 10 November 1999. From these, 11 were immediately discarded either because no dealate (mother) queen was found ($n = 8$) or no sex-ratio bias occurred ($n = 3$). The remaining 24 colonies were reared under laboratory conditions, as described (12). The sex ratio was determined for each colony 1 to 3 weeks after collection from the field. Numerical sex ratio (proportion of males among sexuals) was estimated by sexing at least 100 haphazardly selected sexuals (adult alates + pupae) per colony and calculating the proportion belonging to each sex. Sex investment ratio, estimated as the proportional investment in males among sexuals, was determined for each colony by adjusting the number of males and females by their respective dry weights at the time of mating flights. Mean dry weights of males and females were 8.12 ± 0.68 mg and 2.6 ± 0.3 mg, respectively (26). Because males generally have a higher metabolic rate and, hence, on a per weight basis cost more to produce than females, investment ratios were corrected by converting female/male dry weight ratios (D) to energetic costs ratios (C), with $C = D0.7$ (7).
29. Eggs laid by queens for 24 hours in culture cups before the exchange experiments were collected after removal of the queen. They were incubated with workers for 3 days before they were stored for subsequent genetic analyses. For each colony, seven adult workers and five adult males (if any) were genotyped with one to six microsatellite loci (*Sol-6*, *Sol-11*, *Sol-20*, *Sol-42*, *Sol-49*, and *Sol-55*). These loci have three, seven, eight, nine, seven, and eight alleles, respectively, in the North American population of the fire ant. Primer sequences and amplification conditions are described elsewhere (32). The genotype of the queen and its mate were inferred at each locus on the basis of workers and male offspring genotypes, under the assumption of strict monogyny and monoandry (33). This is straightforward because of the haploidy of males, because, for each locus, a male gives the same allele to all his offspring. Primary sex ratio was assessed by typing 22 to 41 eggs laid per queen at one or two microsatellite loci for which the father had a different allele than that of the queen, so that all fertilized (female) eggs were het-

erozygous. Hence, eggs having either one or two alleles were unambiguously scored as hemizygous males and heterozygous females, respectively. Colonies for which an ambiguity remained were discarded from the analyses.

30. W. R. Tschinkel, *Ecol. Monogr.* **63**, 425 (1993).
31. A. F. G. Bourke, in *Behavioural Ecology: An Evolutionary Approach*, J. R. Krebs, N. B. Davies, Eds. (Blackwell Science, Oxford, 1997), pp. 203–227.
32. M. Krieger, L. Keller, *Mol. Ecol.* **6**, 997 (1997).
33. K. G. Ross, D. J. C. Fletcher, *Behav. Ecol. Sociobiol.* **17**, 349 (1985).

34. We thank M. Chapuisat, C. De Heev, K. Helms, C. Husseheder, and J. Seger for useful comments on the manuscript; C. Roger for help with microsatellites analyses; and the Tournesol exchange program (S.A. and L.P.), the Texas Imported Fire Ant Research and Management Program and the W. M. Keck Center for Behavioral Biology (E.L.V.), the Belgian National Science Foundation (S.A.), and the Swiss National Science Foundation and the "Fondation Herbette" (L.K.) for financial support.

30 April 2001; accepted 20 June 2001

Migratory Movements, Depth Preferences, and Thermal Biology of Atlantic Bluefin Tuna

Barbara A. Block,^{1*} Heidi Dewar,^{1,2} Susanna B. Blackwell,¹ Thomas D. Williams,³ Eric D. Prince,⁴ Charles J. Farwell,³ Andre Boustany,¹ Steven L. H. Teo,¹ Andrew Seitz,³ Andreas Walli,¹ Douglas Fudge³

The deployment of electronic data storage tags that are surgically implanted or satellite-linked provides marine researchers with new ways to examine the movements, environmental preferences, and physiology of pelagic vertebrates. We report the results obtained from tagging of Atlantic bluefin tuna with implantable archival and pop-up satellite archival tags. The electronic tagging data provide insights into the seasonal movements and environmental preferences of this species. Bluefin tuna dive to depths of >1000 meters and maintain a warm body temperature. Western-tagged bluefin tuna make trans-Atlantic migrations and they frequent spawning grounds in the Gulf of Mexico and eastern Mediterranean. These data are critical for the future management and conservation of bluefin tuna in the Atlantic.

The natural history and migratory abilities of Atlantic bluefin tuna (*Thunnus thynnus*) have fascinated mankind for millennia (1). These fish grow to >300 cm and attain masses of 680 kg (2). They are powerful swimmers that range from the tropics to polar latitudes (3) and are renowned for their endothermic physiology (4). Despite a history of exploitation that spans thousands of years, little is known about the spatial dynamics of bluefin tuna movements, depth preferences, or thermal biology.

Atlantic bluefin tuna have been considered overexploited since 1982, and recent catches continue to exceed historical levels (2, 5). The International Commission for the Conservation of Atlantic Tunas (ICCAT) regulates the fishery and currently recognizes

two management units, west and east Atlantic (separated by the 45°W meridian), the latter including the Mediterranean Sea. Larval surveys indicate two major breeding grounds, the Gulf of Mexico and the Mediterranean Sea (2, 3, 6–8). Eastern and western Atlantic bluefin tuna populations are presumed to reach maturity at distinct ages (8–10). The differences in maturity indices, coupled with isolated breeding grounds, suggest that distinct evolutionary units may exist. The west and east Atlantic populations are assumed in ICCAT stock assessments to be mixing at a low level (2). However, conventional tagging data have shown that Atlantic bluefin are capable of making rapid trans-Atlantic crossings (2, 3).

Western Atlantic breeding populations have declined in the past 30 years (2, 5). This has resulted in a reduction in quota for nations that fish this management unit, primarily off the North American coast, and the establishment of recovery plans for the western Atlantic fishery. Critical to a recovery is knowledge of the extent of overlap between the two management units and the level of philopatry to western and eastern breeding grounds (2, 5).

¹Tuna Research and Conservation Center, Stanford University, Hopkins Marine Station, Oceanview Boulevard, Pacific Grove, CA 93950, USA. ²Pfleger Institute of Environmental Research, 1400 North Pacific Street, Oceanside, CA 92054, USA. ³Monterey Bay Aquarium, 886 Cannery Row, Monterey, CA 93940, USA. ⁴National Marine Fisheries Service (NMFS), 75 Virginia Beach Drive, Miami, FL 33149, USA.

*To whom correspondence should be addressed. E-mail: bblock@stanford.edu