

6. M. Jeltsch et al., *Science* **276**, 1423 (1997); S. J. Oh et al., *Dev. Biol.* **188**, 96 (1997).
7. V. Joukov et al., *EMBO J.* **15**, 290 (1996); J. Lee et al., *Proc. Natl. Acad. Sci. U.S.A.* **93**, 1988 (1996).
8. M. G. Achen et al., *Proc. Natl. Acad. Sci. U.S.A.* **95**, 548 (1998); A. Eichmann et al., *Development* **125**, 743 (1998).
9. A. Kaipainen et al., *Proc. Natl. Acad. Sci. U.S.A.* **92**, 3566 (1995); E. Kuk et al., *Development* **122**, 3829 (1996).
10. We generated a null VEGFR-3 allele by introducing a LacZ cassette into the VEGFR-3 locus (71). The 3-kb Kpn I–Not I and Bam HI–Kpn I fragments from 129SV mouse genomic DNA library ( $\lambda$ FIX II; Stratagene) were used as the arms of the targeting vector (Fig. 1). The gene-targeting event was confirmed by Southern (DNA) blot analysis (Fig. 1) (D. J. Dumont et al., data not shown), and two independent embryonic stem cell lines were used to make heterozygous mice through blastocyst injection. Wild-type, heterozygous, and homozygous embryos and mice were identified by Southern blot analysis of yolk sac and tail DNA, respectively.
11. L. Jussila, J. Taipale, K. Alitalo, unpublished data.
12. M. C. Puri et al., *EMBO J.* **14**, 5884 (1995).
13. F. R. Sabin, *Anat. Rec.* **6**, 335 (1912).
14. Embryos were stained for  $\beta$ -Gal (12), photographed, and subsequently dehydrated and embedded in paraffin. Seven-micrometer sections were stained immunohistochemically for PECAM-1 (Pharmingen, San Diego, CA) with TSA Kit (NEN, Boston, MA). Mouse VEGFR-3 [base pairs (bp) 22 to 214; GenBank U73620] and VEGF-C (bp 499 to 656; GenBank U73620) cDNA probes were used for in situ hybridization (9).
15. For a review, see W. Risau, *Nature* **386**, 671 (1997).
16. A. Vecchi et al., *Eur. J. Cell Biol.* **63**, 247 (1994).
17. The presence of VEGFR-3 mRNA in endothelial cells of the dorsal aorta and the yolk sac was confirmed by in situ hybridization (A. Lymboussaki and K. Alitalo, data not shown).
18. J. Rossant, *Circ. Res.* **78**, 349 (1996).
19. S. Hiratsuka et al., *Proc. Natl. Acad. Sci. U.S.A.* **95**, 9349 (1998).
20. J. E. Visvader, Y. Fujiwara, S. H. Orkin, *Genes Dev.* **12**, 473 (1998).
21. L. C. Wang et al., *EMBO J.* **16**, 4374 (1997).
22. P. Carmeliet et al., *Nature* **383**, 73 (1996).
23. L. Wojnowski et al., *Nature Genet.* **16**, 293 (1997).
24. T. N. Sato et al., *Nature* **376**, 70 (1995).
25. H. U. Wang, F.-F. Chen, D. J. Anderson, *Cell* **93**, 741 (1998).
26. M. Oshima, H. Oshima, M. M. Taketo, *Dev. Biol.* **179**, 297 (1996).
27. C. Suri et al., *Cell* **87**, 1161 (1997).
28. P. Lindahl et al., *Science* **277**, 242 (1997).
29. C. T. K. Kuo et al., *Genes Dev.* **11**, 2996 (1997).
30. We thank A. Kaipainen for helpful discussions, E. Koivunen for expert help with the mice, and M. Helanterä, P. Ylikantola, and T. Tainola for technical assistance. Supported by research grants from the Medical Research Council (Canada) and AMGEN (to D.J.D.) and by the Finnish Cancer Organizations, the Finnish Academy, the Sigrid Juselius Foundation, the Finnish State Technology Development Centre, and the European Union Biomed Program.

15 July 1998; accepted 30 September 1998

# Loss of Intraspecific Aggression in the Success of a Widespread Invasive Social Insect

David A. Holway,\* Andrew V. Suarez, Ted J. Case

Despite the innumerable ecological problems and large economic costs associated with biological invasions, the proximate causes of invasion success are often poorly understood. Here, evidence is provided that reduced intraspecific aggression and the concomitant abandonment of territorial behavior unique to introduced populations of the Argentine ant contribute to the elevated population densities directly responsible for its widespread success as an invader. In the laboratory, nonaggressive pairs of colonies experienced lower mortality and greater foraging activity relative to aggressive pairs. These differences translated into higher rates of resource retrieval, greater brood production, and larger worker populations.

Biological invasions threaten the integrity of the world's biota (1, 2). Of the many invading organisms, social insects are among the most harmful: their invasions damage agricultural systems, disrupt natural communities, affect large geographic areas, and are expensive to control (3). Here, we used an experimental approach to investigate the mechanisms underlying the success of a widespread invasive social insect, the Argentine ant (*Linepithema humile*). Experimental approaches are essential to understanding the basis of invasion success (4), but such studies are rare (5).

Ant colonies often have well-defined territorial boundaries, a condition referred to as multicoloniality (6). Multicolonial ants defend their territories aggressively, particularly against conspecifics. Such behavior is thought to limit population density in ants (6, 7), and for animals generally (8), because

territorial defense expends resources, time, and energy that could otherwise be allocated to growth, maintenance, and reproduction (9). Not all ants defend territories. In unicolonial species, for example, intraspecific aggression is reduced, colony boundaries are weak to nonexistent, and supercolonies composed of interconnected nests are the norm (6, 7). Worker populations of unicolonial ants often attain high densities (7, 10, 11), perhaps because unicolonial species are exempt from the costs of defending territories against conspecifics. Numerical advantages, stemming from high population densities, are key to the competitive ability of many ants (12, 13), including invasive species (10, 14).

Native to South America, the Argentine ant has been introduced into areas with Mediterranean and subtropical climates throughout the world, where it displaces native ants (6, 11, 15) and other arthropods (16) and disrupts mutualisms (17). In its introduced range, *L. humile* is highly unicolonial, occupying expansive supercolonies that lack clear behavioral borders (6, 18, 19). In southern

California, for example, intraspecific aggression is rare, even over large spatial scales (>100 km), suggesting the presence of expansive supercolonies (19). In its native range, Argentine ants exhibit pronounced intraspecific aggression over small spatial scales (<100 m) and maintain colony structures more closely resembling those of multicolonial ants (19). This variation in the occurrence of intraspecific aggression among colonies permits a direct test of the mechanisms responsible for the elevated population densities typical of unicolonial ants. Moreover, this variation provides an unparalleled opportunity to assess experimentally how territoriality may limit population size (20).

We tested the relationship between intraspecific aggression and population size by rearing pairs of lab colonies that either did or did not exhibit intraspecific aggression. To do this, we sampled workers and queens from spatially separate nests in southern California (21). We then set up 44 experimental colonies (22), each consisting of three queens, 500 workers, and <50 brood pieces. Using plastic tubing, we connected colonies into pairs via a common foraging arena. Colony pairs were placed into three experimental categories on the basis of the origin of each nest and whether they exhibited intraspecific aggression (23). Categories included an aggressive treatment (pairs that exhibited aggression and were collected from different sites), a nonaggressive treatment (pairs that did not exhibit aggression and were collected from different sites), and a control (colonies from the same site). For each colony pair, we quantified intraspecific aggression (24), worker mortality, foraging activity, resource retrieval rates, and productivity (25).

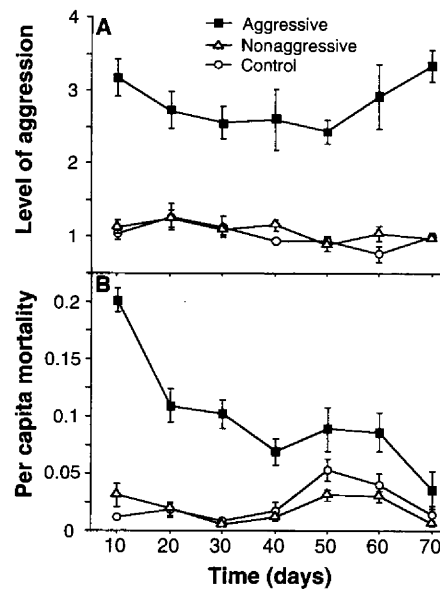
Throughout the experiment, workers from the aggressive treatment group remained aggressive (often fighting to the death), whereas workers from the nonaggressive treatment and control groups rarely showed any sign of

Department of Biology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA.

\*To whom correspondence should be addressed. E-mail: dholway@biomail.ucsd.edu

## REPORTS

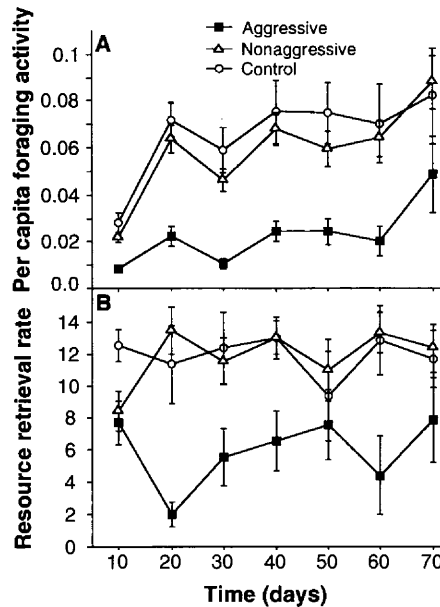
aggressive behavior (Fig. 1A). As a result, per capita mortality was higher in the aggressive treatment group than in the nonaggressive treatment or control groups (Fig. 1B). In the aggressive treatment group, mortality declined after the first sampling period as territorial boundaries became fixed; workers in this group guarded tube openings in the foraging arena leading back to their nest, presumably to prevent raids from the opposing colony. By contrast, in the nonaggressive treatment and control groups, workers and even queens moved between the nest containers



**Fig. 1.** Mean levels of aggression and per capita mortality ( $\pm$ SE). (A) Argentine ant workers in the aggressive treatment group remained consistently more aggressive than workers in the nonaggressive treatment or control groups. MANOVA for all three categories:  $\lambda$  (Wilks') = 0.200,  $F_{2,18} = 66.012$ ,  $P < 0.0001$ . Pairwise MANOVAs: aggressive versus nonaggressive,  $\lambda = 0.147$ ,  $F_{1,13} = 57.829$ ,  $P < 0.001$ ; aggressive versus control,  $\lambda = 0.142$ ,  $F_{1,10} = 78.736$ ,  $P < 0.001$ ; nonaggressive versus control,  $\lambda = 0.899$ ,  $F_{1,13} = 1.461$ ,  $P > 0.1$ . A simple behavioral assay was used to estimate aggression between pairs of workers from nests within each colony pair (24). (B) Elevated aggression in the aggressive treatment group translated into higher per capita mortality at the level of the colony relative to that in the nonaggressive treatment or control groups. MANOVA for all three categories:  $\lambda = 0.128$ ,  $F_{2,18} = 58.135$ ,  $P < 0.0001$ . Pairwise MANOVAs: aggressive versus nonaggressive,  $\lambda = 0.129$ ,  $F_{1,13} = 81.296$ ,  $P < 0.001$ ; aggressive versus control,  $\lambda = 0.152$ ,  $F_{1,10} = 55.898$ ,  $P < 0.001$ ; nonaggressive versus control,  $\lambda = 0.973$ ,  $F_{1,13} = 0.330$ ,  $P > 0.1$ . Dead workers were removed from each colony pair at 10-day intervals and counted. Measures of mortality are for each colony pair, because it was impossible to assign dead workers within each pair to a specific colony. Per capita mortality was estimated as the number of dead workers divided by the total number of workers.

within each colony pair. Fusions between nests (cases in which queens from both nests moved in together) occurred in 67% (10/15) of colony pairs in the nonaggressive treatment and control groups combined.

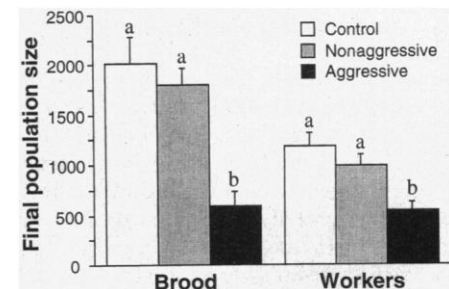
Differences in intraspecific aggression shown in Fig. 1 affected both foraging behavior and colony growth. In the aggressive treatment group, per capita foraging activity was reduced relative to the nonaggressive treatment or control groups (Fig. 2A). As a result, aggressive colony pairs harvested resources less quickly than did colony pairs in



**Fig. 2.** Mean per capita foraging activity and absolute rates of resource retrieval ( $\pm$ SE). (A) Fewer Argentine ant workers in the aggressive treatment group foraged than did those in the nonaggressive treatment and control groups. MANOVA for all three categories:  $\lambda = 0.365$ ,  $F_{2,18} = 15.624$ ,  $P < 0.0001$ . Pairwise MANOVAs: aggressive versus nonaggressive,  $\lambda = 0.268$ ,  $F_{1,13} = 35.539$ ,  $P < 0.001$ ; aggressive versus control,  $\lambda = 0.291$ ,  $F_{1,10} = 24.415$ ,  $P < 0.001$ ; nonaggressive versus control,  $\lambda = 0.964$ ,  $F_{1,13} = 0.485$ ,  $P > 0.1$ . A worker was considered foraging when it was in the foraging arena. Per capita foraging activity is defined as the mean number of foragers (from 4 to 10 daily counts within each 10-day interval) relative to the total number of workers present in each colony pair. (B) Argentine ant workers in the aggressive treatment group retrieved resources at a slower rate than did workers from the nonaggressive treatment or control groups. MANOVA for all three categories:  $\lambda = 0.564$ ,  $F_{2,18} = 6.948$ ,  $P < 0.01$ . Pairwise MANOVAs: aggressive versus nonaggressive,  $\lambda = 0.493$ ,  $F_{1,13} = 13.389$ ,  $P < 0.01$ ; aggressive versus control,  $\lambda = 0.529$ ,  $F_{1,10} = 8.885$ ,  $P < 0.05$ ; nonaggressive versus control,  $\lambda = 0.999$ ,  $F_{1,13} = 0.001$ ,  $P > 0.1$ . At 10-day intervals, rates of resource retrieval were measured by placing a weigh boat containing 15 dead fruit flies into each foraging arena and counting the number of flies removed (dependent variable) from the weigh boat after 15 min.

the nonaggressive treatment or control groups (Fig. 2B). Reduced access to food limited colony growth for aggressive colony pairs. Relative to colony pairs in the aggressive treatment group, colony pairs in the nonaggressive treatment and control groups produced more than three times the amount of brood and supported about twice the number of workers (Fig. 3). The smaller worker populations in the aggressive treatment group can be attributed to a combination of lower brood production and higher worker mortality relative to the nonaggressive treatment and control groups (26).

These results help to explain the underlying basis of the Argentine ant's widespread ecological success. The strong competitive ability of this species results from high worker densities rather than the competitive prowess of individual workers. In California, Argentine ant workers are smaller than workers of many of the native ants they displace; as a result, Argentine ants win interference contests against other species primarily through numerical advantages (14). In addition, the higher foraging rates seen in the nonaggressive treatment group suggest that an absence of intraspecific aggression may enhance exploitative ability as well. As shown here, the lack of intraspecific aggression typical of introduced populations allows spatially separate colonies to fuse and to achieve higher worker densities compared to colonies that exhibit intraspecific aggression. The high levels of intraspecific aggression common to native populations of Argentine ants (19) probably



**Fig. 3.** Mean numbers ( $\pm$ SE) of brood and workers at the conclusion of the experiment. Colony pairs in the aggressive treatment group had fewer brood (that is, eggs, larvae, and pupae) at the end of the experiment relative to colony pairs in the nonaggressive treatment or control groups (one-way ANOVA:  $F_{2,18} = 14.171$ ,  $P < 0.001$ ; bars with different letters are statistically different using Dunnett's test). Because brood production could not be sampled without excessively disturbing the colonies, we counted brood only at the end of the experiment. Colony pairs in the aggressive treatment group had fewer workers at the end of the experiment relative to colony pairs in the nonaggressive treatment or control groups (one-way ANOVA:  $F_{2,18} = 8.355$ ,  $P < 0.005$ ; bars with different letters are statistically different using Dunnett's test).

limit colony size and reduce competitive ability. Support for this hypothesis comes from Argentina, where *L. humile* occurs in species-rich assemblages of ants (19), completely unlike what is observed throughout its introduced range (11, 14, 15).

The Argentine ant may also benefit from the absence of coevolved natural enemies in its introduced range, as is often suggested for the success of invasive species (2, 27). For example, host-specific phorid fly parasitoids affect the competitive ability of many ecologically dominant ants (28), including the Argentine ant and other invasive species (29). The lack of natural enemies in the Argentine ant's introduced range probably works in conjunction with the loss of intraspecific aggression to give Argentine ants a competitive edge over native species.

Our findings also illustrate the social plasticity of introduced populations of Argentine ants. Pairs in the nonaggressive treatment group were formed from colonies up to 80 km apart, yet often fused and were as productive as control colony pairs composed of ants collected from the same site. These data demonstrate the extent of the Argentine ant's unicoloniality in terms of colony-level productivity and indicate that Argentine ants can behave as a single supercolony over large spatial scales.

The underlying basis of the loss of intraspecific aggression in the Argentine ant remains unknown. Ross and colleagues found that changes in the social behavior of the imported fire ant (*Solenopsis invicta*) resulted from inbreeding after introduction into the southeastern United States (30). Although introduced populations of Argentine ants are less genetically variable than native populations (19), the role of genetic similarity in moderating fighting behavior still deserves careful study. For example, the loss of intraspecific aggression typical of introduced populations of this species may result from a lack of stimuli due to high genetic relatedness between colonies, or an innate loss of aggression due to a breakdown in nestmate discriminatory ability.

Our results provide experimental evidence of how territorial behavior (and interference competition more generally) affects population size through reductions in per capita efficiencies in competitive ability (31). These results have population-level implications because once Argentine ant colonies fuse into supercolonies, worker density becomes the most ecologically meaningful measure of population size. Although the lack of territoriality in unicolonial ants is believed to explain their high population densities (7, 10, 11), this is the first experimental demonstration of this hypothesis. Moreover, the consequences of reduced intraspecific aggression probably extend to other social

insect invasions. Most invasive ants, and some invasive termites, exhibit reduced nestmate recognition, muted intraspecific aggression, and unicoloniality to varying degrees (6, 32). These results also demonstrate the value of studying behavioral characteristics of invasive species, an often neglected component in the study of biological invasions.

# References and Notes

1. C. S. Elton, *The Ecology of Invasions by Animals and Plants* (Wiley, New York, 1958); H. A. Mooney and J. A. Drake, Eds., *Ecology of Biological Invasions of North America and Hawaii* (Springer-Verlag, New York, 1986); J. A. Drake et al., Eds., *Biological Invasions: A Global Perspective* (Wiley, Chichester, UK, 1989); P. M. Vitousek et al., *Am. Sci.* **84**, 468 (1996).
2. S. L. Pimm, *The Balance of Nature? Ecological Issues in the Conservation of Species and Communities* (Univ. of Chicago Press, Chicago, 1991).
3. B. S. Vinson, Ed., *Economic Impact and Control of Social Insects* (Praeger, New York, 1986); R. K. Vandermeer, K. Jaffe, A. Cedeno, Eds., *Applied Myrmecology: A World Perspective* (Westview, Boulder, CO, 1990); M. L. Winston, *Killer Bees: The African Honey Bee in the Americas* (Harvard Univ. Press, Cambridge, MA, 1991); D. F. Williams, Ed., *Exotic Ants: Biology, Impact, and Control of Introduced Species* (Westview, Boulder, CO, 1994).
4. J. Diamond and T. J. Case, in *Community Ecology*, J. Diamond and T. J. Case, Eds. (Harper and Row, New York, 1986), pp. 64–79; P. Kareiva, *Ecology* **77**, 1651 (1996); G. J. Vermeij, *Biol. Conserv.* **78**, 3 (1996).
5. K. Petren, D. T. Bolger, T. J. Case, *Science* **259**, 354 (1993); C. M. D'Antonio, *Ecology* **74**, 83 (1993).
6. B. Hölldobler and E. O. Wilson, *The Ants* (Harvard Univ. Press, Cambridge, MA, 1990).
7. ———, *Naturwissenschaften* **64**, 8 (1977).
8. J. L. Brown, *Wilson Bull.* **81**, 293 (1969); S. D. Fretwell and H. L. Lucas, *Acta Biotheor.* **19**, 16 (1970).
9. B. Hölldobler and C. J. Lumsden, *Science* **210**, 732 (1980); N. B. Davies and A. I. Houston, in *Behavioral Ecology: An Evolutionary Approach*, J. R. Krebs and N. B. Davies, Eds. (Blackwell Scientific, Oxford, ed. 2, 1984), pp. 148–169; F. L. Carpenter, *Am. Zool.* **27**, 227 (1987); J. Stamps, *Adv. Study Behav.* **23**, 173 (1994).
10. S. D. Porter and D. A. Savignano, *Ecology* **71**, 2095 (1990); T. E. Macom and S. D. Porter, *Ann. Entomol. Soc. Am.* **89**, 535 (1996).
11. D. A. Holway, *Oecologia* **116**, 252 (1998).
12. B. Hölldobler, *ibid.* **52**, 208 (1983).
13. J. F. Lynch, E. C. Balinsky, S. G. Vail, *Ecol. Entomol.* **5**, 353 (1980); J. Fellers, *Ecology* **68**, 1466 (1987); E. S. Adams, *Anim. Behav.* **39**, 321 (1990).
14. B. D. Tremper, thesis, University of California, Berkeley (1976); D. A. Holway, *Ecology*, in press.
15. J. M. Erickson, *Psyche* **78**, 257 (1971); P. S. Ward, *Hilgardia* **55**, 1 (1987); J. D. Majer, in *Exotic Ants: Biology, Impact, and Control of Introduced Species*, D. F. Williams, Ed. (Westview, Boulder, CO, 1994), pp. 163–173; K. Human and D. M. Gordon, *Oecologia* **105**, 405 (1996); M. E. Cammel, M. J. Way, M. R. Paiva, *Insectes Soc.* **43**, 37 (1996); A. V. Suarez, D. T. Bolger, T. J. Case, *Ecology* **79**, 2041 (1998).
16. F. R. Cole et al., *Ecology* **73**, 1313 (1992).
17. M. J. Way, *Annu. Rev. Entomol.* **8**, 307 (1963); W. Bond and P. Slingsby, *Ecology* **65**, 1031 (1984).
18. W. Newell and T. C. Barber, *USDA Bur. Entomol. Bull.* **122** (1913); G. P. Markin, *J. Kans. Entomol. Soc.* **41**, 511 (1968); G. P. Markin, *Ann. Entomol. Soc. Am.* **63**, 1238 (1970).
19. A. V. Suarez, N. D. Tsutsui, D. A. Holway, T. J. Case, *Biol. Invasions*, in press.
20. A. Watson, *Nature* **215**, 1274 (1967); I. J. Patterson, *Ardea* **68**, 53 (1980); A. Watson and R. Moss, *ibid.*, p. 103.
21. We collected ants for experimental colonies from two sites at each of three cities in southern California: Encinitas (E1 and E2), La Jolla (L1 and L2), and Temecula (T1 and T2). Temecula is about 50 km north of Encinitas, which is 20 km north of La Jolla. Sites within each city were >1 km apart.
22. We reared experimental colonies in plastic nest containers (30 cm by 14 cm by 8 cm) lined with flunon and tanglefoot to prevent ants from escaping. Each nest container contained two nest chambers (glass test tubes half full of water and stopped with cotton) covered with aluminum foil to keep them dark. Every other day, we fed experimental colonies scrambled eggs, fresh crickets, and 25% sugar water. Colonies were reared under a light-dark cycle (12 hours each) at 24°C. Before the onset of the experiments, we left the experimental colonies in an undisturbed state for several weeks. At the start of the experiments, food was placed exclusively in a foraging arena (a plastic container the size of the nest containers) connecting colonies within each pair.
23. Each colony pair was a unique combination of experimental colonies; pairings were as follows. Aggressive treatment:  $n = 6$ ; E2 versus L1, T2 versus T1, L1 versus T1, E1 versus T1, E2 versus T1, L2 versus T1. Nonaggressive treatment:  $n = 9$ ; E1 versus L2, E1 versus T2, E1 versus E2, E1 versus L1, L2 versus T2, T2 versus L1, L2 versus E2, T2 versus E2, L1 versus L2. Control:  $n = 6$ ; E1 versus E1, E2 versus E2, L1 versus L1, L2 versus L2, T1 versus T1, T2 versus T2. In the aggressive treatment, we maintained two E1 versus T1 colony pairs and used the mean response of these two colony pairs for all analyses. The average distances between the source colonies used within each experimental pair were about 45 km in the aggressive treatment, 44 km in the nonaggressive treatment, and 20 m in the control. For all statistical analyses, we used the response of colony pairs as data points.
24. We measured intraspecific aggression using standardized fighting trials (19). For each colony pair, we randomly selected a single worker from each nesting container and placed them together in a 2-dram glass vial with flunon-coated sides for 10 min. We scored the behavioral interactions that ensued in the following categories in order of escalating aggression: ignore = 0 (contacts between individuals where neither ant showed any interest in the other), touch = 1 (contacts that included prolonged antennation), avoid = 2 (contacts that resulted in one or both ants retreating in opposite directions), aggression = 3 (lunging, biting, and pulling legs or antennae), or fight = 4 (prolonged aggression between individuals). For each pair of colonies, we recorded the highest level of aggression observed in each of five trials and used the mean of these trials for all statistical comparisons.
25. We performed four separate multivariate analyses of variance (MANOVAs) to compare aggression levels, per capita mortality, per capita foraging activity, and resource retrieval rates among the three experimental categories in this study. For each analysis, we performed an overall MANOVA comparing the three categories (independent variables) across the seven time periods (dependent variables). Within each overall MANOVA, we then performed three pairwise MANOVAs and used the  $P$  values from the univariate  $F$  tests to test for differences among each pair of treatments. Reported  $P$  values are Bonferroni-adjusted (within each overall MANOVA) for three comparisons. See Figs. 1 and 2 for details regarding the dependent variables used in each analysis. All statistical analyses were performed using JMP (SAS Institute, version 3, 1996).
26. At 24°C, eggs laid at the start of the study would have developed into workers in about 54 days, within the duration of the study. Natural worker life-spans may exceed 6 months [see (18)].
27. D. M. Lodge, in *Biotic Interactions and Global Change*, P. M. Kareiva, J. G. Kingsolver, R. B. Huey, Eds. (Sinauer, Sunderland, MA, 1993), pp. 367–387; S. D. Porter, D. F. Williams, R. S. Patterson, H. J. Fowler, *Environ. Entomol.* **26**, 373 (1997).
28. D. H. Feener Jr., *Science* **214**, 815 (1981); ——— and B. V. Brown, *Annu. Rev. Entomol.* **42**, 73 (1996).
29. M. R. Orr, S. H. Seike, W. W. Benson, L. E. Gilbert, *Nature* **373**, 292 (1995); M. R. Orr and S. H. Seike, *Oecologia*, in press.
30. K. G. Ross and L. Keller, *Annu. Rev. Ecol. Syst.* **26**, 631

- (1995); K. G. Ross, E. L. Vargo, L. Keller, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 3021 (1996).
31. T. J. Case and M. E. Gilpin, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3073 (1974); T. J. Case, *Ecology* **56**, 3 (1975).
32. L. Passera, in *Exotic Ants: Biology, Impact, and Control of Introduced Species*, D. F. Williams, Ed. (Westview, Boulder, CO, 1994), pp. 23–43; T. G. Shelton and J. K. Grace, *Environ. Entomol.* **26**, 632 (1997).
33. We thank J. French, R. Gonzalez, J. Hee, and F. Nekouee for help with the lab work, and M. Levenstein

and J. Kadonaga for providing *Drosophila*. J. Bradbury, M. Dantzker, F. Dyer, K. Marchetti, T. Price, N. Tsutsui, and P. Ward provided valuable discussion and read earlier versions of the manuscript. Supported by USDA postdoctoral fellowship 97-35302-4920 (D.A.H.), the Canon National Parks Science Scholars Program (A.V.S.), and NSF grant DEB-9610306 (T.J.C.).

28 July 1998; accepted 24 September 1998

## Haldane's Rule in Taxa Lacking a Hemizygous X

Daven C. Presgraves and H. Allen Orr

Haldane's rule states that species hybrids of the XY sex are preferentially sterile or inviable. In all taxa known to obey this rule, the Y is inert and X-linked genes show full expression in XY individuals. Until recently, all theories of Haldane's rule depended on this hemizygosity. A test of Haldane's rule in animals lacking a hemizygous sex—mosquitoes having two functional sex chromosomes in both sexes—reveals that these species show Haldane's rule for sterility but not inviability. A related group having a "normal" hemizygous X obeys Haldane's rule for both sterility and inviability. These results support the faster male and dominance theories of Haldane's rule.

Mosquitoes of the genus *Aedes* have single-locus sex determination: Although females are XX and males are XY, both X and Y chromosomes carry complete homologous sets of genes and differ only at a single locus (or small chromosome region) specifying sex. Cytological work reveals that the X and Y, which recombine throughout their lengths, are morphologically indistinguishable (1). Genetic analysis of more than 60 sex-linked, visible, electrophoretic and DNA markers confirms that the Y carries homologous alleles at all loci studied (1, 2). Y gene activity has been confirmed in at least nine species of *Aedes* (1–3). Mosquitoes of the genus *Anopheles*, on the other hand, possess degenerate Y chromosomes and X-linked genes that show normal hemizygous expression and sex-linked patterns of inheritance (4).

The fact that *Aedes* lacks a hemizygous X, whereas *Anopheles* possesses one, allows several novel tests of the leading theories explaining Haldane's rule (5–7). Recent work suggests that Haldane's rule has two causes. The first, the so-called dominance theory, posits that the genes causing hybrid problems are mostly recessive (8–10). If so, XY individuals will suffer the full effects of all X-linked alleles causing hybrid problems, whereas XX individuals will partly mask such alleles in the heterozygous state. Consequently, the XY sex will suffer more severe hybrid problems than will the XX sex, and Haldane's rule results (8–10). Experiments with *Drosophila* suggest that dominance ex-

plains Haldane's rule for hybrid inviability (11–13). Dominance may also contribute to Haldane's rule for sterility, although the evidence here is less direct (12–14).

A second force, faster male evolution, may also cause Haldane's rule for sterility (6). Because male and female fertility typi-

cally involve different loci, Haldane's rule might simply reflect a faster rate of divergence of genes involved in male than in female reproduction. If so, hybrid male sterility would tend to arise before hybrid female sterility, yielding Haldane's rule in taxa with XY males. Several causes of faster male evolution have been suggested (6), the most popular positing that sexual selection drives especially rapid evolution of male-expressed genes (6, 15). Recent experiments suggest that faster male evolution may give rise to Haldane's rule for sterility, at least in *Drosophila* (12, 16). The faster male theory cannot, however, be extended to hybrid inviability, because genes affecting viability almost always affect both sexes (lethal mutations within species almost invariably kill both sexes) (6, 12, 16). The faster male theory also cannot be extended to explain hybrid sterility in taxa such as birds and butterflies, in which heterogametic females are preferentially sterile (6, 10).

The consensus view of Haldane's rule is, therefore, simple: Haldane's rule for inviability appears to be caused by dominance alone, whereas in taxa with heterogametic males, Haldane's rule for sterility appears to be caused by both dominance and faster male evolution (7, 12, 16, 17).

The contrast between *Aedes* and *Anopheles* provides nearly ideal material for testing this view. These theories predict that: (i) If the faster male theory is correct, *Aedes* should

**Table 1.** *Aedes* hybridizations. B, both sexes affected; M, males affected; F, females affected; N, neither sex affected. Dashes indicate no data or that data did not match criteria (18). Some hybridizations are reviewed in (23); a full list of references is available from the authors.

| Species pair              |                              | Cross |       |
|---------------------------|------------------------------|-------|-------|
| A                         | B                            | A × B | B × A |
| Hybrid sterility          |                              |       |       |
| <i>Ae. zoosophas</i>      | <i>Ae. hendersoni</i>        | B     | B     |
| <i>Ae. zoosophas</i>      | <i>Ae. brelandi</i>          | —     | B     |
| <i>Ae. zoosophas</i>      | <i>Ae. triseriatus</i>       | M     | M     |
| <i>Ae. triseriatus</i>    | <i>Ae. brelandi</i>          | M     | N     |
| <i>Ae. triseriatus</i>    | <i>Ae. hendersoni</i>        | M     | N     |
| <i>Ae. sollicitans</i>    | <i>Ae. taeniorhynchus</i>    | B     | B     |
| <i>Ae. taeniorhynchus</i> | <i>Ae. nigromaculatus</i>    | B     | B     |
| <i>Ae. s. malayensis</i>  | <i>Ae. s. polynesiensis</i>  | M     | —     |
| <i>Ae. cooki</i>          | <i>Ae. pseudoscutellaris</i> | M     | —     |
| <i>Ae. cooki</i>          | <i>Ae. p. Niuafo'o</i>       | M     | N     |
| <i>Ae. s. malayensis</i>  | <i>Ae. s. katharensis</i>    | M     | —     |
| <i>Ae. mariae</i>         | <i>Ae. zammitii</i>          | M     | M     |
| <i>Ae. mariae</i>         | <i>Ae. phoeniciae</i>        | M     | B     |
| <i>Ae. zammitii</i>       | <i>Ae. phoeniciae</i>        | B     | B     |
| Hybrid inviability        |                              |       |       |
| <i>Ae. albopictus</i>     | <i>Ae. aegypti</i>           | B     | B     |
| <i>Ae. albopictus</i>     | <i>Ae. seatoi</i>            | B     | B     |
| <i>Ae. albopictus</i>     | <i>Ae. pseudoalbopictus</i>  | B     | B     |
| <i>Ae. seatoi</i>         | <i>Ae. pseudoalbopictus</i>  | B     | B     |
| <i>Ae. pernotatus</i>     | <i>Ae. hebrideus</i>         | —     | M     |
| <i>Ae. communis</i>       | <i>Ae. churchillensis</i>    | B     | —     |
| <i>Ae. zoosophas</i>      | <i>Ae. brelandi</i>          | B     | —     |
| <i>Ae. aegypti</i>        | <i>Ae. simpsoni</i>          | —     | B     |
| <i>Ae. kesseli</i>        | <i>Ae. alcasidi</i>          | F     | —     |

Department of Biology, University of Rochester, Rochester, NY 14627, USA.