Natural Selection and the Reinforcement of Mate Recognition

Megan Higgie,[1]* Steve Chenoweth,[2] Mark W. Blows[1]

Natural selection on mate recognition may often contribute to speciation, resulting in reproductive character displacement. Field populations of *Drosophila serrata* display reproductive character displacement in cuticular hydrocarbons when sympatric with *Drosophila birchii*. We exposed field sympatric and allopatric populations of *D. serrata* to experimental sympatry with *D. birchii* for nine generations. Cuticular hydrocarbons of field allopatric *D. serrata* populations evolved to resemble the field sympatric populations, whereas field sympatric *D. serrata* populations remained unchanged. Our experiment indicates that natural selection on mate recognition resulted in the field pattern of reproductive character displacement.

A direct role for natural selection in the generation of reproductive isolation during speciation is highly controversial (*1–6*). Natural selection may rapidly increase divergence in mate recognition between sympatric populations of speciating animal taxa by selecting against hybridization between heterotypic individuals. The reinforcement of mate recognition by this process will result in the pattern of reproductive character displacement (*3*), where sympatric populations of closely related species have diverged in mate recognition to a greater extent than allopatric populations.

Reproductive character displacement has been found in a diverse range of taxa (*3–9*). However, the pattern of reproductive character displacement does not constitute direct evidence for natural selection reinforcing mate recognition (*10*) because it may be caused by a number of other processes (*3*, *6*). In particular, it has yet to be shown that a trait which displays reproductive character displacement in the field is heritable and responds to natural selection on mate recognition within the context of an experiment that excludes other possible causes of the displacement. Here, we use a natural selection experiment (*11*) to demonstrate the role of natural selection in the generation of a field pattern of reproductive character displacement.

Drosophila serrata and *D. birchii* have different but overlapping distributions and habitat associations along the east coast of Australia (*12*). The two species are sexually isolated by pheromones composed of cuticular hydrocarbons (CHCs) (*13*, *14*) and hybrids are viable and fertile (*12*, *13*). Species in which a single reproductive trait is largely responsible for mate recognition provide the best systems for studying reproductive character displacement (*15*). In this species pair, mate recognition and CHC profile are highly genetically correlated, with genetic variation in CHCs accounting for over 70% of the genetic variation in mate recognition between the two species (*14*). Selection on mate recognition is therefore likely to result in changes in CHC profile. The CHCs of *D. serrata* display reproductive character displacement, changing abruptly at the sympatry-allopatry border (Fig. 1), although the pattern is still confounded with geographic distribution as in many other examples (*16*).

To test whether natural selection on mate recognition generated the reproductive character displacement in the CHCs of natural populations of *D. serrata*, we exposed three field sympatric and three field allopatric populations of *D. serrata* to experimental sympatry with *D. birchii* for nine generations (*17*). We predicted that if the field pattern of reproductive character displacement was the result of natural selection on mate recognition in sympatry, the CHCs of field allopatric populations would evolve in experimental sympatry, whereas those of field sympatric populations would not. The CHCs of experimental sympatry and control lines were assayed after selection ended (*18*). A canonical discriminant analysis (*19*) was used to display the relation between the experimental populations in multivariate CHC space (Fig. 2). The first two canonical variates (CV1 and CV2), accounting for 94.9% and 2.4% of the variation, respectively, were analyzed in univariate split-plot analyses of variance (*20*). The interaction between the treatment of experimental sympatry and whether the populations were originally allopatric or sympatric in the field tested if evolution in experimental sympatry had occured differentially in field allopatric and sympatric populations. Paired *t* tests were used to determine if the presence of an interaction was a consequence of evolution in field allopatric populations, rather than in field sympatric populations.

Drosophila serrata females from field allopatric populations exposed to experimental sympatry evolved toward the field sympatric control populations in multivariate CHC space (Fig. 2). Univariate analysis of variance (ANOVA) conducted on female CV1 and CV2 indicated interactions between the treatment of experimental sympatry and whether the population was allopatric or sympatric in the field [CV1, $F(1,4) = 8.199$, $P = 0.046$; CV2, $F(1,4) = 7.519$, $P = 0.052$]. Evolution occurred in field allopatric populations (paired *t* tests: CV1, $t_2 = 4.273$, $P = 0.051$; CV2, $t_2 = -5.086$, $P = 0.037$), but not in field sympatric populations (CV1, $t_2 = -1.609$, $P = 0.249$; CV2, $t_2 = -0.525$, $P = 0.652$). *Drosophila serrata* males from two of the three field allopatric populations exposed to experimental sympatry also evolved toward the field sympatric control populations. The males of the third field allopatric population (Wollongong) evolved in the same direction along CV1 as the first two populations but in the opposite direction on CV2 (Fig. 2). Univariate ANOVA conducted on male CV1 indicated an interaction between exposure to experimental sympatry

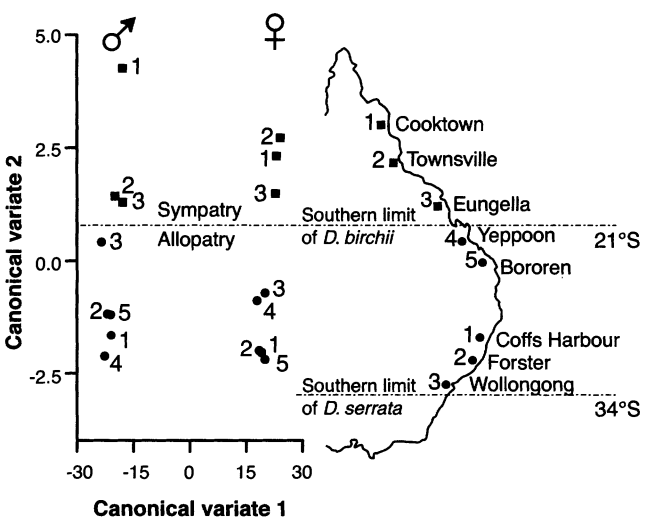

Fig. 1. Reproductive character displacement in the cuticular hydrocarbons of *D. serrata*. The map of eastern Australia shows the distributions of *D. serrata* and *D. birchii* as well as the positions of three sympatric (■) and five allopatric (●) populations of *D. serrata*. Population means are from the first two canonical variates from a canonical discriminant analysis conducted using the CHC data from the control populations from the selection experiment plus the two additional populations (4 and 5).

[1]Department of Zoology and Entomology, University of Queensland, St. Lucia 4072, Australia. [2]Faculty of Environmental Sciences, Griffith University, Nathan 4111, Australia.

*To whom correspondence should be addressed: E-mail: MHiggie@zoology.uq.edu.au

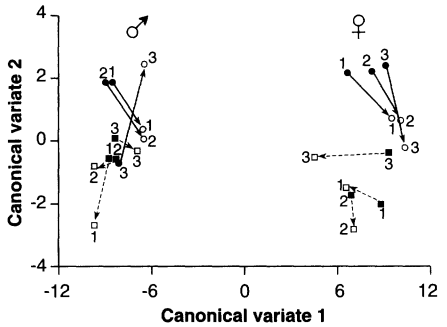

Fig. 2. The effect of natural selection on the cuticular hydrocarbons of *D. serrata* after nine generations of experimental sympatry with *D. birchii*. Evolutionary responses are indicated from control populations (closed symbols) to experimental sympatry populations (open symbols) of field allopatric populations (circles connected by solid arrow) and field sympatric populations (squares connected by dashed arrow). Numbers refer to localities given in Fig. 1.

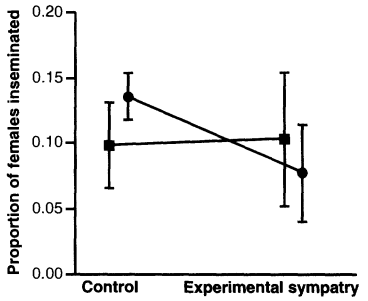

Fig. 3. The effect of experimental sympatry on the efficiency of field sympatric and allopatric *D. serrata* males in inseminating *D. serrata* females. Means and 95% confidence intervals are based on the three field allopatric (●) and sympatric (■) populations.

and whether the population was allopatric or sympatric in the field [$F(1,4) = 7.056$, $P = 0.057$], which was a consequence of evolution in field allopatric populations ($t_2 = -8.628$, $P = 0.013$) and no change in field sympatric populations ($t_2 = -0.362$, $P = 0.752$). Male CV2 displayed no interaction as a consequence of the Wollongong population.

Experimental sympatry resulted in the evolution of the CHCs of both sexes of *D. serrata* originating from field allopatric populations. In general, the response of both sexes was to evolve toward the field sympatric control populations. The CHCs of field sympatric populations did not respond to the presence of *D. birchii* in experimental sympatry, indicating that the reproductive character displacement displayed in Fig. 1 was a consequence of natural selection on mate recognition under field conditions.

Reproductive character displacement evolved within nine generations, indicating that there was strong selection on mate recognition. To determine whether selection on mate recognition operated either prezygotically during courtship or postzygotically after hybrids were formed, we conducted two experiments. First, the frequency of successful hybridization in our experimental sympatry treatments was determined (*21*) and was found to be very low, suggesting that little selection was applied postzygotically. Second, prezygotic selection was assessed by determining the efficiency of *D. serrata* males in inseminating *D. serrata* females in experimental sympatry (*22*). Experimental sympatry differentially affected the efficiency of field sympatric and field allopatric males in inseminating females [$F(1,2) = 64.39$, $P = 0.015$] (Fig. 3). Field allopatric males inseminated significantly fewer females in experimental sympatry than in the allopatric controls (paired t test: $t_2 = 5.179$, $P = 0.035$), with nearly 50% fewer *D. serrata* females inseminated in the presence of *D. birchii*. In contrast, the number of females inseminated by field sympatric males was unaffected by experimental sympatry ($t_2 = 1.220$, $P = 0.347$). Selection on mate recognition therefore operated during courtship, rather than after the production of hybrid individuals with low fitness, to generate the reproductive character displacement in CHC profile.

Although natural selection in our laboratory environment operated during courtship, prezygotic selection for mate recognition may not be as intense under natural conditions if adult densities are lower and the larval substrate is not limited to a single patch (bottle). The form that selection takes under field conditions to result in reproductive character displacement in this system remains to be evaluated. Furthermore, this experiment does not indicate whether natural selection on mate recognition in sympatry was a component of the historical speciation event between *D. serrata* and *D. birchii*, but rather indicates how rapidly the mate recognition system of populations may evolve when confronted with the presence of a closely related group. The large number of cases of apparent reproductive character displacement in field populations across diverse taxonomic groups (*3–9*) suggests that natural selection on mate recognition may be a major component of the evolution of mate recognition in many animals (*1*).

References and Notes

1. T. Dobzhansky, *Genetics and the Origin of Species* (Columbia Univ. Press, New York, ed. 3, 1951), pp. 206–211
2. R. Butlin, *Trends Ecol. Evol.* **2**, 8 (1987).
3. D. J. Howard, in *Hybrid Zones and the Evolutionary Process*, R. G. Harrison, Ed. (Oxford Univ. Press, New York, 1993), pp.46–69.
4. R. Butlin, in *Speciation and the Recognition Concept*, D. M. Lambert, H. G. Spencer, Eds. (John Hopkins Univ. Press, Baltimore, 1995), pp. 327–366.
5. M. A. Noor, *Am. Nat.* **149**, 1156 (1997).
6. ———, *Heredity* **83**, 503 (1999).
7. G.-P Sætre *et al.*, *Nature* **387**, 589 (1997).
8. M. A. Noor, *Nature* **375**, 674 (1995).
9. H. D. Rundle, D. Schluter, *Evolution* **52**, 200 (1998).
10. The five experimental criteria (3) required to establish that reproductive character displacement was a consequence of natural selection on mate recognition are as follows: (i) heterotypic matings occur, (ii) there is selection against hybridization, (iii) the displacement is perceptible to the other sex, (iv) the trait is heritable and responds to selection, and (v) other, particularly ecological, processes have not resulted in the displacement. Only the first two criteria have been commonly addressed in specific cases (*3*, *9*). Experiments to address criteria (iv) and (v) have been attempted in sticklebacks (*9*, *23*) and in the *Drosophila pseudoobscura*/*D. persimilis* pair of species (*8*, *24*).
11. Many previous experimental studies (*25*) have used laboratory-derived stocks in artificial selection experiments to investigate the plausibility of reinforcement without the context of a naturally occurring pattern of reproductive character displacement.
12. F. J. Ayala, *Evolution* **19**, 538 (1965).
13. M. W. Blows, *Am. Nat.* **151**, 538 (1998).
14. ———, R. A. Allan, *Am. Nat.* **152**, 826 (1998).
15. W. F. Blair, *Q. Rev. Biol.* **39**, 334 (1964).
16. R. Butlin, *Trends Ecol. Evol.* **10**, 432 (1995).
17. Populations were founded by 10 to 20 field-caught inseminated females and were maintained at a census size of >100 individuals for between 3 to 12 months in the laboratory at 25°C before the beginning of the study. A treatment (experimental sympatry) and control population were established for each of the six localities. Experimental sympatry was applied by maintaining three culture bottles per population, each bottle containing 20 *D. serrata* females and 20 *D. serrata* males from the respective locality and 20 *D. birchii* females and 20 *D. birchii* males. The control populations were maintained in the same fashion without *D. birchii*, ensuring that the population size of *D. serrata* was the same between experimental sympatry and control groups. All flies were 1-day-old virgins, so that mate choice for *D. serrata* individuals in experimental sympatry began in the presence of *D. birchii*. The percentage of *D. birchii* males that emerged in experimental sympatry populations each generation was only 4.6% on average, because *D. birchii* has a lower innate rate of increase (*26*) and lower productivity (*27*) than *D. serrata*. Therefore, *D. birchii* were added to experimental sympatry populations each generation to bring the percentage of each species back to 50%.
18. At the 10th generation, five replicate vials, each containing one virgin *D. serrata* male and two virgin females, were established for each of the 12 experimental populations. After 5 days, the females were separated into their own individual vials. Two female and two male offspring were prepared from each female for gas chromatograph (GC) analysis (*14*). Mixed culturing of *Drosophila* species may change CHCs (*28*), so culturing *D. serrata* individuals in isolation for one generation ensured that only genetic change was detected between experimental sympatry and control populations. Ten individuals of each sex from the two additional populations not included in the selection experiment (4 and 5 in Fig. 1) were also prepared for the GC at this time.
19. Analysis of the hydrocarbon data set was conducted following established protocols using logcontrasts (*14*). A canonical discriminant analysis (CDA) was conducted on the mean logcontrasts of each sex for each of the 12 populations, because this level represented the independent application of sympatry or allopatry in the field.
20. The model for this experimental design is a split-plot and is given by $Y_{ijk} = \mu + P_i + A/S_j + PA/S_{ij} + T_k + PT_{ik} + A/ST_{jk} + PA/ST_{ijk}$, where P is geographic population, A/S is whether a population was allopatric or sympatric to *D. birchii* in the field, and T is the treatment of experimental sympatry or control. The P by T and P by A/S by T interactions may be considered nonexistent in split-plot ANOVA because populations (P) are random replicates, and so they were pooled to create an error term for the split plot with increased degrees of freedom (*29*) to test the A/S by T interaction.
21. We used the same experimental design as that in the selection experiment but with four replicate bottles per population: two bottles in which the *D. serrata* females and *D. birchii* males were sterile and two in which the *D. serrata* males and *D. birchii* females were sterile. Sterilization was achieved by exposing 1-day-old virgin flies to a 0.2-kGy dose of radiation over 15 min from a ^{60}Co source. Sterilization ensured that any female producing

viable offspring in a bottle was a consequence of an interspecific mating. After 6 days in the bottles, females were placed singly into vials, which were scored for larval activity after another 6 days. Only 2 of the 480 fertile females in the experiment produced viable offspring; one in a field allopatric population (Forster) and one in a field sympatric population (Eungella). This frequency of hybridization is about four times greater than that achieved with single heterotypic pairs (*13*).

22. Single 1-day-old *D. serrata* males were placed in bottles with either 20 1-day-old virgin *D. serrata* females (controls) or 20 1-day-old *D. serrata* females and 20 1-day-old sterilized *D. birchii* females and males (experimental sympatry). Five replicate bottles of each treatment were set up for each of the six geographic populations. After 2 days in the bottle, females were placed singly in vials, and after another 6 days were scored for larval activity, indicating the number of *D. serrata* females that had been inseminated in the 2-day period. The mean proportion of *D. serrata* females inseminated for each population was analysed in a split-plot ANOVA (*20*).
23. H. D. Rundle, L. Nagel, J. W. Boughman, D. Schluter, *Science* **287**, 306 (2000).
24. K. F. Koopman, *Evolution* **4**, 135 (1950).
25. W. R. Rice, E. E. Hostert, *Evolution* **47**, 1637 (1993).
26. L. C. Birch, T. Dobzhansky, P. O. Elliot, R. C. Lewontin, *Evolution* **17**, 72 (1963).
27. F. J. Ayala, *Genetics* **51**, 527 (1965).
28. Y.-K. Kim, H. R. Koepfer, L. Ehrman, *Behav. Genet.* **26**, 27 (1996).
29. C. R. Hicks, *Fundamental Concepts in the Design of Experiments* (Saunders College, New York, 1993), p. 268.
30. We thank C. Moore and R. Diocares for GC technical advice and R. Brooks, P. Cassey, J. Endler, A. Hoffmann, I. Owens, C. Sgro, and two anonymous reviewers for comments on the manuscript. Supported by a grant from the Australian Research Council to M.W.B.

11 May 2000; accepted 6 July 2000

Invasive Plants Versus Their New and Old Neighbors: A Mechanism for Exotic Invasion

Ragan M. Callaway* and Erik T. Aschehoug†

Invading exotic plants are thought to succeed primarily because they have escaped their natural enemies, not because of novel interactions with their new neighbors. However, we find that *Centaurea diffusa*, a noxious weed in North America, has much stronger negative effects on grass species from North America than on closely related grass species from communities to which *Centaurea* is native. *Centaurea*'s advantage against North American species appears to be due to differences in the effects of its root exudates and how these root exudates affect competition for resources. Our results may help to explain why some exotic species so successfully invade natural plant communities.

Exotic plants threaten the integrity of agricultural and natural systems throughout the world. Many invasive species are not dominant competitors in their natural systems, but competitively eradicate their new neighbors. One leading theory for the exceptional success of invasive plants is that they have escaped the natural enemies that hold them in check, freeing them to utilize their full competitive potential. This perspective provides the theoretical framework for the widespread practice of introducing natural enemies as biological controls, which also are exotic, to suppress invasive plants (*1*). Plant communities are widely thought to be "individualistic," composed primarily of species that have similar adaptations to a particular physical environment (*2*, *3*). With few exceptions (*4*–*7*), plant communities are not thought to consist of coevolved species, nor to possess stable properties determined by plant-plant interactions. Here, we argue that some invasive plants may succeed because they bring novel mechanisms of interaction to natural plant communities.

We compared the competitive effects of an invasive Eurasian forb, *Centaurea diffusa* (diffuse knapweed), on three bunchgrass species that coexist with *C. diffusa* in Eurasia with the effects of *C. diffusa* on three bunchgrass species from North America that have similar morphologies and sizes, each of which is closely related to one of the Eurasian grass species. Seeds of *C. diffusa*, *Festuca ovina*, *Koeleria laerssenii*, and *Agropyron cristatum* were collected within an area of several hectares in the southern foothills of the Caucasus Mountains in the Republic of Georgia. Seeds of *F. idahoensis*, *K. cristata*, and *Pseudoroegneria spicata* were collected from grasslands in the northern Rocky Mountains in Montana. Until recently, *Pseudoroegneria* was included in the genus *Agropyron*. Each of the grass species made up more than 10% of the total cover at its respective site. At the study site in the Caucasus, the cover of *C. diffusa* was less than 1%, whereas at the Montana site, the cover of *C. maculosa* (which is closely related to *C. diffusa*) was 10 to 90%. Each of the seven species was planted alone and in all pairwise grass-*Centaurea* combinations. All combinations were grown in sand and mixed with activated carbon (*8*, *9*).

Centaurea diffusa had much stronger negative effects on North American species than it had on Eurasian species. When grown with *Centaurea*, the biomass of North American grasses decreased 85.7 ± 0.3%; whereas in Eurasian species, biomass decreased by only 50.0 ± 4.7% (Fig. 1) (*10*). Correspondingly, none of the North American grass species (nor all species analyzed collectively) had a significant competitive effect on the biomass of *C. diffusa*, but the Eurasian species *K. laerssenii*, and all Eurasian species analyzed collectively, significantly reduced *C. diffusa* biomass (Fig. 2) (*11*). *Centaurea diffusa* had no effect on the amount of ^{32}P acquired by Eurasian grass species (*12*), but significantly reduced ^{32}P uptake of all North American species (Fig. 3) (*13*). Correspondingly, North American grasses had no competitive effects on ^{32}P uptake of *C. diffusa*, but all Eurasian species demonstrated strong negative effects on the amount of ^{32}P acquired by *C. diffusa* (Fig. 4) (*14*).

Activated carbon was added to ameliorate chemical effects (*8*), and it had contrasting effects on the interactions between *C. diffusa* and grass species from the different continents. The biomass of two North American species, *F. idahoensis* and *P. spicata*, when grown with *C. diffusa*, increased significantly in soil mixed with activated carbon; the overall effect of car-

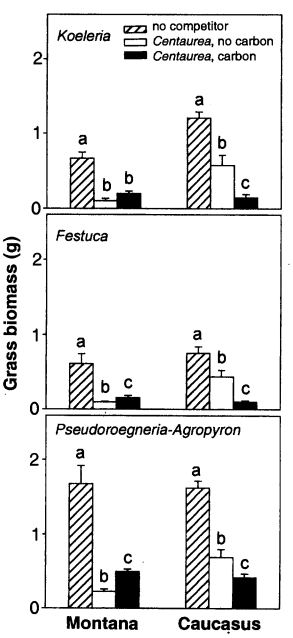

Fig. 1. Total biomass for related Eurasian and North American bunchgrass species grown alone, or with the invasive plant, *C. diffusa*, either with or without activated carbon in the soil. Error bars represent S.E.M. Means with different letters were significantly different in pairwise comparisons.

Division of Biological Sciences, The University of Montana, Missoula, MT 59812, USA.

*To whom correspondence should be addressed. E-mail: callaway@selway.umt.edu

†Present address: The Nature Conservancy, 201 Mission Street, San Francisco, CA 94105, USA.