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2 authors, including:

obtained from the MP2 calculations (23). Earlier microwave studies revealed that adenine has a large inertial defect (24). Although this is consistent with our results, it does not uniquely determine the source of the nonplanarity.

The simplicity of the above spectrum results from the relatively small size of the molecule and the fact that there is only a single isomer of adenine produced by thermal evaporation. In contrast, cytosine is known to have a number of low-energy isomeric forms (25). Although it is well established that there are at least keto and enol forms of cytosine, there is still some uncertainty regarding which form(s) of the latter can be observed (26). As shown in Fig. 4, two different enol forms have been proposed (25), associated with the directionality of the O-H bond.

The spectrum of the $NH_2(SS)$ region of cytosine (solvated in He droplets) is shown in Fig. 4. It consists of three bands, even though a single isomer of cytosine would only have a single band in this spectral region. The implication is that there are three isomers of cytosine present in the He droplet environment. Nonetheless, an unambiguous assignment of these bands on the basis of the vibrational frequencies alone is difficult, given their small spacing relative to the accuracy of the corresponding ab initio calculations. It is evident from Fig. 4 that the field dependences of these three bands are distinctly different. When compared with the ab initio VTMAs for the various isomers of cytosine (Table 1), the assignment of the spectrum becomes clear. Namely, in order of increasing vibrational frequency, the bands are assigned to the keto, cis-enol, and trans-enol isomers. Further evidence in support of this assignment comes from the other X-H stretches of these isomers (Table 1). In all cases, there is excellent agreement between the experimental and calculated VTMAs.

The largest deviation (10°) occurs for the trans-enol O-H stretch, which could be an indication of mode coupling in this case, although the signal-to-noise ratio for this band is rather low, making the data somewhat less accurate. It is noteworthy that the assignment based on the VTMAs puts the cis-enol isomer at a slightly lower frequency than the trans-enol form, which is exactly the opposite of the ab initio prediction. Indeed, the frequencies for these two isomers are so similar as to preclude an assignment based on the frequencies alone. Indeed, these two bands were not even resolved in the previous argon matrix isolation study of this system (26).

Our results for adenine show that the molecule is nonplanar, with the NH_2 group tilted ~20° out-of-plane. As pointed out elsewhere (27), it was generally believed (28) that nucleic acid bases were planar, or at least that the biological consequences of nonplanarity were weak. This point of view changed with the observation of abundant interstrand amino-group contacts in B-DNA crystal structures, which appear to be stabilized by amino-group pyramidalization and interstrand bifurcated hydrogen bonds (27). Although the ab initio calculations provide considerable evidence for the nonplanarity of the bases, it was noted in 1999 that "clear, direct experimental evidence about the nonplanarity of isolated bases is still missing due to the resolution of the available experimental techniques" (27). This situation has now changed and it will be interesting to study other systems, including guanine, for which ab initio calculations suggest the out-of-plane angle is anomalously large (27).

The results reported here for cytosine show how VTMAs can also be used to assign complex spectra arising from the presence of multiple tautomers. The method will be particularly useful in the study of water clusters with biomolecules, where the location of the water molecule can easily be determined by recording the VTMAs for the corresponding H-bonded and free O-H stretching vibrations. It is also encouraging that the number of VTMAs increases with the number of atoms in the molecule ($3N - 6$ in principle), so that more structure information is available for the larger systems where it is needed most.

References and Notes
1. B. C. Dian, A. Longarte, T. S. Zwier, *Science* **296**, 2369 (2002).
2. D. W. Pratt, *Science* **296**, 2347 (2002).
3. D. R. Borst, J. R. Roscioli, D. W. Pratt, *J. Phys. Chem. A* **106**, 4022 (2002).
4. T. S. Zwier, *J. Phys. Chem. A* **105**, 8827 (2001).
5. L. C. Snoek, R. T. Kroemer, J. P. Simons, *Phys. Chem. Chem. Phys.* **4**, 2130 (2002).
6. A. Dkhissi *et al.*, *J. Phys. Chem. A* **104**, 9785 (2000).
7. E. Nir *et al.*, *Phys. Chem. Chem. Phys.* **4**, 732 (2002).
8. M. Mons *et al.*, *J. Phys. Chem. A* **106**, 5088 (2002).
9. J. Michl, E. W. Thulstrup, *Spectroscopy with Polarized Light: Solute Alignment by Photoselection in Liquid Crystals, Polymers, and Membranes*, (VCH, Weinheim, Germany, 1995), pp. 150–189.
10. D. Marsh, M. Müller, F.-J. Schmitt, *Biophys. J.* **78**, 2499 (2000).
11. A. Holmén, *J. Phys. Chem. A* **101**, 4361 (1997).
12. J. G. Radziszewski *et al.*, *J. Am. Chem. Soc.* **118**, 10275 (1996).
13. K. Nauta, R. E. Miller, *Science* **283**, 1895 (1999).
14. K. K. Lehmann, G. Scoles, *Science* **279**, 2065 (1998).
15. Helium nanodroplets were formed by expanding high-purity helium gas from 60 atm through an orifice 5 μm in diameter (cooled to ~23 K) into vacuum, resulting in a beam of droplets with a mean diameter of ~3 nm (~3000 atoms). These droplets passed through an oven containing a low vapor pressure (between 10^{-6} and 10^{-5} torr) of the nucleic acid base of interest. The gas-phase molecules were captured and solvated by the droplets when collisions between them occurred. The seeded droplets then passed through an IR laser [an F-center laser (16)], where the solvated molecule was vibrationally excited. Relaxation of the excited molecule led to the evaporation of several hundred He atoms and caused a depletion of the droplet beam, which was detected with a bolometer (16). The IR spectrum was obtained by scanning the laser through the various vibrational bands and recording the laser-induced decrease in the He droplet beam intensity.
16. R. E. Miller, *Science* **240**, 447 (1988).
17. M. Hartmann, R. E. Miller, J. P. Toennies, A. F. Vilesov, *Phys. Rev. Lett.* **75**, 1566 (1995).
18. W. Kong, *Int. J. Mod. Phys. B* **15**, 3503 (2001).
19. C. Plützer, E. Nir, M. S. De Vries, K. Kleinermanns, *Phys. Chem. Chem. Phys.* **3**, 5466 (2001).
20. M. J. Frisch *et al.*, *Gaussian 98* (Gaussian, Pittsburgh, PA, 1998).
21. K. Nauta, D. T. Moore, P. L. Stiles, R. E. Miller, *Science* **292**, 481 (2001).
22. C. Callegari, K. K. Lehmann, R. Schmied, G. Scoles, *J. Chem. Phys.* **115**, 10090 (2001).
23. S. K. Mishra, M. K. Shukla, P. C. Mishra, *Spectrochim. Acta A* **56**, 1355 (2000).
24. J. S. Kwiatkowski, R. J. Bartlett, W. B. Person, *J. Am. Chem. Soc.* **110**, 2353 (1988).
25. P. Ü. Civcir, *J. Mol. Struct.* (*Theochem.*) **532**, 157 (2000).
26. M. Szczesniak *et al.*, *J. Am. Chem. Soc.* **110**, 8319 (1988).
27. P. Hobza, J. Sponer, *Chem. Rev.* **99**, 3247 (1999).
28. S. Kawahara, T. Uchimaru, K. Taira, M. Sekine, *J. Phys. Chem. A* **106**, 3207 (2002).
29. Supported by NSF grant CHE-99-87740. We thank W. Kong for providing the program for calculating the orientation distributions.

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Sperm-Female Coevolution in *Drosophila*

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Rapid evolution of reproductive traits has been attributed to sexual selection arising from interaction between the sexes. However, little is known about the nature of selection driving the evolution of interacting sex-specific phenotypes. Using populations of *Drosophila melanogaster* selected for divergent sperm length or female sperm-storage organ length, we experimentally show that male fertilization success is determined by an interaction between sperm and female morphology. In addition, sperm length evolution occurred as a correlated response to selection on the female reproductive tract. Giant sperm tails are the cellular equivalent of the peacock's tail, having evolved because females evolved reproductive tracts that selectively bias paternity in favor of males with longer sperm.

Male reproductive traits appear to evolve more rapidly than other types of character (1). For example, DNA sequence comparisons reveal that male-derived molecules involved in reproduction exhibit a high level of divergence among members of the primate lineage
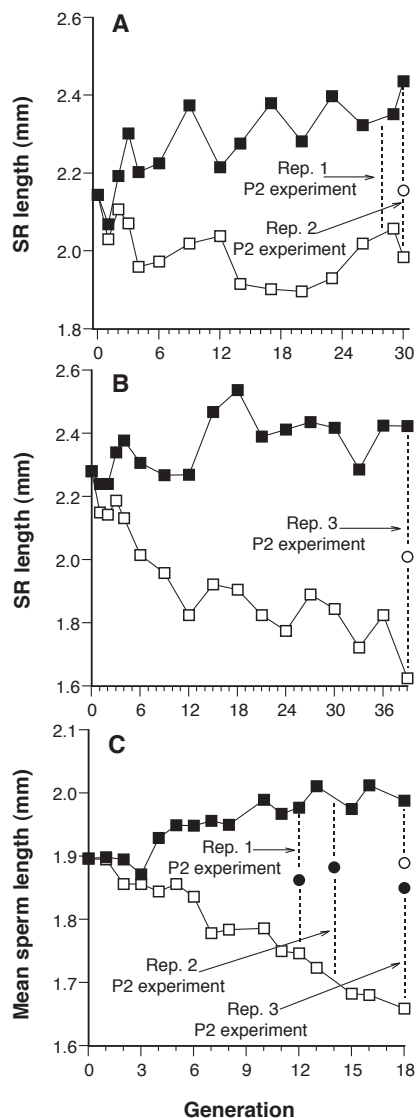
leading to humans (*2*), between mouse and rat (*3*), among marine invertebrates (*4*), and between closely related *Drosophila* species (*5*). Positive Darwinian selection is the driving force behind this rapid evolution (*2–5*). Models to explain this pattern include sexually antagonistic coevolution and postcopulatory sexual selection and are largely based on male-female interaction (*6–9*). In line with this view, a recent study has demonstrated positive Darwinian selection driving rapid evolution of mammalian female reproductive proteins as well (*10*). Despite the growing number of studies illustrating this pattern at the molecular and genetic level and theory-based explanations (*6, 7, 9*), few studies have identified the interacting sex-specific phenotypes or have experimentally shown the nature of selection driving their rapid evolutionary divergence (*11*).

Sperm are the most diverse cell type, evolving so rapidly that their external morphology and ultrastructure provide reliable cues for distinguishing taxa and their phylogenetic relationships (*12*). Sperm tail length variation is of particular interest, as comparative studies on diverse taxa have found positive relationships between sperm size and the risk of sperm competition (*13, 14*). A pattern of correlated evolution between sperm length and certain dimensions of the female reproductive tract has been identified in taxa as diverse as birds (*15*), butterflies (*16*), and fruit flies (*17*). Collectively, these studies strongly implicate postcopulatory sexual selection mediated by a component of cryptic female choice referred to as "sperm choice" (*18*). This process, defined as nonrandom paternity biases resulting from female morphology, physiology, or behavior that occur after coupling, has proved difficult to demonstrate (*13, 18*). We have experimentally explored the adaptive importance of sperm length in *D. melanogaster* and provide evidence for its evolution by female sperm choice. *Drosophila* species provide a valuable system for investigating sperm evolu-

tion, because variation in sperm length within the genus exceeds variation in the rest of the animal kingdom. At one extreme, *D. bifurca* have evolved gigantic sperm that are 58.290 ± 0.66 mm long, or about 20 times the total body length of males (*19*).
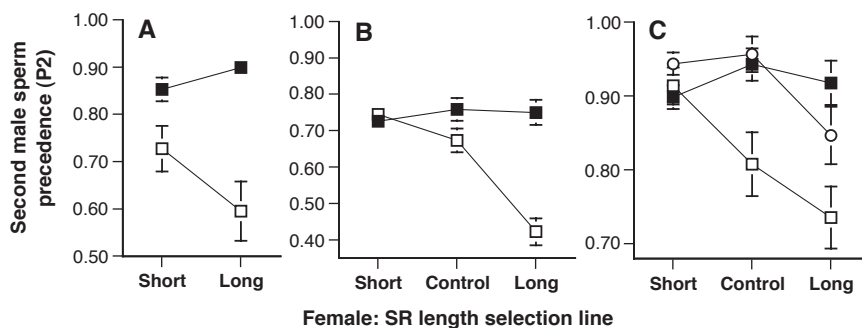
We established independent populations of *D. melanogaster* that were selected for either increased or decreased sperm length or the length of the females' primary sperm-storage organ, the seminal receptacle (SR) (Fig. 1). The sperm length selection experiment had a single replicate, and the SR length selection experiment had two replicates (each replicate consisting of a long, short, and control population). Next, sperm competition experiments (*N* = 3 replicates) were conducted in which females from the SR selection lines were each initially mated to a standard "competitor" male (bearing an eye color mutation that allowed offspring paternity to be assigned) and then remated to a male from one of the sperm length selection lines. The proportion of progeny sired by the second male (P2) was then determined. The first replicate had four experimental treatments: short-sperm males and long-sperm males were each competed within short-SR and long-SR females from the first replicate of the SR selection experiment (Fig. 2A). The second replicate had six experimental treatments: the same design as the first replicate with the addition of control-SR females, again using females from the first replicate of the SR selection experiment (Fig. 2B). The third replicate had nine experimental treatments: the same design as the second replicate with the addition of control-sperm males, with females coming from the second replicate of the SR selection experiment (Fig. 2C). Because males and females were all derived from independent selection lines, sperm competition outcomes could not be influenced by unforeseen coevolved traits between the sexes. Finally, within the two SR length selection replicates, sperm length was determined

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Fig. 1. Responses to bidirectional selection on length of (**A** and **B**) the female's seminal receptacle (replicates 1 and 2, respectively) and (**C**) sperm. Selection line: open squares, short; open circles, control; solid squares, long. Populations from which females (A, B) and males (C) were derived for each of the three replicate sperm competition experiments are indicated by dotted lines. Sperm length of the "competitor" males against which sperm-selection line males were competed is also indicated (solid circles). SR length selection was performed as described (*27*), except that females were mated multiply and selection was intermittent after the fourth generation. For sperm selection, sperm from 45 males per line per generation were measured as described (*35*); within each line, progeny from the 15 males with the longest or shortest sperm were selected. Control lines were maintained under identical conditions.

Fig. 2. Results of three replicate experiments to determine the proportion of progeny (mean ± SE) sired by males from the sperm-length selection lines within females from the SR-length selection lines. Males: open squares, short-sperm selection line; open circles, control-sperm selection line; solid squares, long-sperm selection line. (**A** and **B**) Replicates 1 and 2, respectively, females from first replicate of SR-length selection; (**C**) replicate 3, females from second replicate of SR-length selection.

after 37 and 42 generations, respectively, to discern any correlated response in males to selection on the female's reproductive tract.

Sperm length and SR length both responded to directional selection (Fig. 1), resulting in statistically highly significant increases and decreases in both traits relative to control populations (20). Sperm length exhibited a realized heritability of 0.478 ± 0.286, and SR length exhibited realized heritabilities of 0.366 ± 0.270 and 0.414 ± 0.266 in the two respective replicates. These populations were used in sperm competition experiments to determine the adaptive importance of variation in sperm and SR length and the level of interaction between these sex-specific traits in determining differential male fertilization success. Interpretation of the sperm competition experimental results is based on analysis of paternity for 43,031 offspring from 1044 females (21).

All three replicates of the sperm competition experiment revealed a strong interaction between male and female traits (Fig. 2 and Table 1). First, males from populations with longer sperm had a fertilization success equal to or better than that of males with shorter sperm. Second, and most notably, we consistently found statistically significant SR length × sperm length interaction effects determining male fertilization success. Thus, the performance of males with different sperm lengths was influenced by the SR length of females within which males were competing. In contrast, the female line influence on variation in P2 was inconsistent, being significant in only one of three replicates. The significant interaction between the sexes may explain why studies that considered only female morphology and not sperm length, or vice versa, have found no effect on P2 (22, 23).

Long-sperm males did not perform better or worse than short-sperm males within short-SR females in any replicate (post hoc Scheffe's F tests: rep. 1, $P = 0.25$; rep. 2, $P = 0.68$; rep. 3, $P = 0.75$). In contrast, long-sperm males sired a significantly greater proportion of progeny than did short-sperm males within control-SR females (rep. 2, $P < 0.05$; rep. 3, $P < 0.01$), and this difference was even more pronounced within long-SR females (rep. 1, $P < 0.001$; rep. 2, $P < 0.01$; rep. 3, $P < 0.001$). As the length of a female's SR increased, so did the value of long sperm among potential sires (Fig. 2). Length of the female's SR thus represents the mechanical determinant of postcopulatory female "sperm choice" (13, 18) based on the sperm length of males in D. melanogaster.

A separate experiment showed that results of the P2 experiment were not attributable to variation in the number of sperm transferred by males. Long-sperm males transferred the fewest sperm, although differences were not significant (mean ± SE: long-sperm males, 1428 ± 65, $N = 10$; short-sperm males, 1584 ± 120, $N = 10$; control-sperm males, 1546 ± 290, $N = 5$) as indicated by analysis of variance (ANOVA) ($F_{2,22} = 0.42$, $P = 0.66$). Further, two-way ANOVA revealed no interaction effect between male and female selection lines on the number of sperm transferred ($F_{1,16} = 2.29$, $P = 0.15$).

Sperm size may influence their position in the SR. Within the SR of all *Drosophila* species, the sperm generally appear to be straightened out rather than coiled (17). In *D. melanogaster*, a small and well-organized group of sperm heads can be observed, 6 hours after copulation, near the proximal end of the SR with their tails extending distally (24). These sperm, by virtue of their location, presumably take precedence over sperm residing more distally within the SR. We spec-

ulate that "being the right size" offers some advantage to sperm in occupying this superior position.

The demonstrated relationship between SR length and sperm use (Fig. 2) is predicted to generate linkage disequilibrium between genes for the female preference and the male ornament, resulting in the genetic correlation assumed by good genes and runaway sexual selection models (25). We tested this prediction by examining correlated responses in sperm length within the SR-length selection lines. Each female had ample opportunity to mate with multiple males before producing offspring for each subsequent generation. Thus, maintenance of genetic covariation between SR length and sperm length could arise through female sperm choice.

Sperm length increased significantly in both long-SR selection lines relative to control-SR lines (ANOVA: rep. A, $F_{2,42} = 32.12$, $P < 0.0001$; rep. B, $F_{2,42} = 8.99$, $P < 0.001$), but showed no significant change in the short-SR selection lines (Fig. 3) [relationships between sperm length and male body size were not significant (20)]. This result is likely attributable to postcopulatory sexual selection rather than to pleiotropy or genetic linkage. Results of the sperm precedence experiments indicate that short SRs do not discriminate among sperm within the natural distribution of lengths, whereas long SRs do discriminate against shorter sperm (Fig. 2). The observed pattern of evolutionary change in sperm length within the SR selection lines is precisely what one would predict from the results of the sperm competition experiments. No such pattern is predicted as a consequence of pleiotropy or linkage. We suggest that the process of female "sperm choice," as demonstrated here for *D. melanogaster*, underlies the marked divergence in sperm length throughout the genus *Drosophila* and other taxa. If true, then postcopulatory sexual se-

Table 1. Analysis of variance and covariance of second male sperm precedence (P2). df, degrees of freedom; MS, type III mean square.

Source	df	MS	F statistic	P
Replicate 1				
Female line	1	0.063	0.47	0.4963
Male line	1	2.408	18.01	<0.0001
Female line × Male line	1	0.534	3.99	<0.05
Error	132	0.134		
Replicate 2				
Female line	2	1.502	9.63	<0.0001
Male line	1	4.125	26.44	<0.0001
Female line × Male line	2	2.244	14.39	<0.0001
Error	495	0.156		
Replicate 3				
Female line	2	0.008	0.08	0.9223
Male line	2	0.529	5.29	<0.01
Remating interval	1	5.9×10^{-6}	5.9×10^{-5}	0.9939
Female line × Male line	4	0.437	4.37	<0.005
Female line × Remate	2	0.077	0.77	0.4614
Male line × Remate	2	0.352	3.52	<0.05
Error	395	0.100		
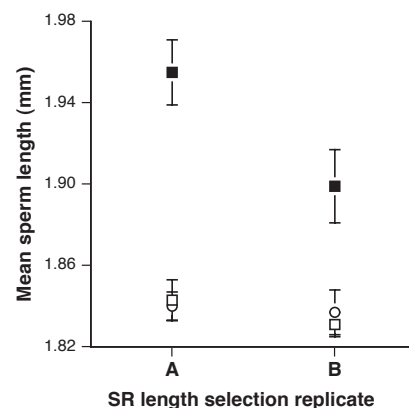
Fig. 3. Correlated response of sperm length (mean ± SE) to selection on length of the female's SR (open squares, short-SR line; open circles, control-SR line; solid squares, long-SR line). $N = 15$ males per line.

lection clearly can favor sperm quality (e.g., length) at the expense of sperm quantity, even when males have limited resources for gamete production (26).

Although we now understand what drives sperm length evolution, we do not know what is driving the evolution of SR length. Nonetheless, this trait offers an exceptionally tractable system for studying the evolution of a female preference and of male-female interactions. The functional relationship between the female preference and the corresponding male ornament is unambiguous, the preference and ornament are both easy to quantify, the macroevolutionary pattern of coevolution between the preference and ornament has been established (17), costs of relative expression of each have been quantified (19, 23, 26), and each is amenable to genetic analysis and artificial selection (27).

Our results are consistent with several models developed to explain the evolution of female mate preferences. Linkage disequilibrium between the female preference and male ornament is consistent with the Fisherian runaway process and "good genes" models (28). Also consistent with good genes models, recent studies have suggested a link between male condition and sperm quality (29), including sperm length (30). Next, interactions between the sexes are rife with conflict in *D. melanogaster* (31) and the coevolution of sperm and SR length may be sexually antagonistic, as has been suggested for sperm length and sperm-storage tubule length in birds (15). Finally, data reported here refute predictions of two sexual selection models as applied to this system. First, the "direct benefits" model (28) cannot apply, as the long sperm tails are not absorbed by females and have not evolved to serve a post-fertilization function (32). Second, the "sensory exploitation" model (28) is not applicable, as phylogenetic analysis reveals a pattern of correlated evolution between the female preference and male trait (17) rather than a pattern of the male trait evolving in response to a preexisting female bias.

The sperm-female coevolution demonstrated here has important implications for diversification and speciation. Rapid morphological divergence of sperm has been reported for numerous taxa, including primates (33). Such divergence has been shown to drive correlated divergence of important life history traits (19, 26). Further, as sperm morphology and sperm usage by females are central to successful reproduction, their divergence will likely contribute to reproductive isolation between populations and the formation of new species (1, 7, 34).

References and Notes

1. W. J. Swanson, V. D. Vacquier, *Nature Rev. Genet.* **3**, 137 (2002).
2. G. J. Wyckoff, W. Wang, C.-I. Wu, *Nature* **403**, 304 (2000).
3. K. A. Sutton, M. F. Wilkinson, *J. Mol. Evol.* **45**, 579 (1997).
4. V. D. Vacquier, *Science* **281**, 1995 (1998).
5. W. J. Swanson, A. G. Clark, H. M. Waldrip-Dail, M. F. Wolfner, C. F. Aquadro, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 7375 (2001).
6. W. R. Rice, *Nature* **381**, 232 (1996).
7. G. A. Parker, L. Partridge, *Philos. Trans. R. Soc. London Ser. B* **353**, 261 (1998).
8. A. G. Clark, D. J. Begun, T. Prout, *Science* **283**, 217 (1999).
9. S. Gavrilets, *Nature* **403**, 886 (2000).
10. W. J. Swanson, Z. Yang, M. F. Wolfner, C. F. Aquadro, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 2509 (2001).
11. G. Arnqvist, L. Rowe, *Evolution* **56**, 936 (2002).
12. B. G. M. Jamieson, *The Ultrastructure and Phylogeny of Insect Spermatozoa* (Cambridge Univ. Press, Cambridge, 1987).
13. L. W. Simmons, *Sperm Competition and Its Evolutionary Consequences in the Insects* (Princeton Univ. Press, Princeton, NJ, 2001).
14. C. W. LaMunyon, S. Ward, *Proc. R. Soc. London Ser. B* **269**, 1125 (2002).
15. J. V. Briskie, R. Montgomerie, T. R. Birkhead, *Evolution* **51**, 937 (1997).
16. M. J. G. Gage, *Proc. R. Soc. London Ser. B* **258**, 247 (1994).
17. S. Pitnick, T. A. Markow, G. S. Spicer, *Evolution* **53**, 1804 (1999).
18. T. R. Birkhead, *Evolution* **54**, 1057 (2000).
19. S. Pitnick, T. A. Markow, G. S. Spicer, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 10614 (1995).
20. G. T. Miller, S. Pitnick, data not shown.
21. Female and male body sizes were measured. Initial analyses of P2 by analysis of covariance (ANCOVA), with female and male lines as main factors and male and female sizes as covariates, revealed that size and its interaction with other variables were never significant and inclusion of body sizes never improved model fit. Entering the variable "remating interval" as a covariate was only justified in the analysis for the third replicate experiment.
22. E. H. Morrow, M. J. G. Gage, *Proc. R. Soc. London Ser. B* **268**, 2281 (2001).
23. G. T. Miller, S. Pitnick, *J. Evol. Biol.*, in press.
24. S. Pitnick, unpublished data.
25. T. C. M. Bakker, A. Pomiankowski, *J. Evol. Biol.* **8**, 129 (1995).
26. S. Pitnick, *Am. Nat.* **148**, 57 (1996).
27. G. T. Miller, W. T. Starmer, S. Pitnick, *Heredity* **87**, 25 (2001).
28. M. Andersson, *Sexual Selection* (Princeton Univ. Press, Princeton, NJ, 1994).
29. A. Rakitin, M. M. Ferguson, E. A. Trippel, *Can. J. Fish. Aquat. Sci.* **56**, 2315 (1999).
30. L. W. Simmons, J. S. Kotiaho, *Evolution* **56**, 1622 (2002).
31. S. Pitnick, F. García-González, *Proc. R. Soc. London Ser. B*, **269**, 1821 (2002).
32. T. L. Karr, S. Pitnick, *Nature* **379**, 405 (1996).
33. M. J. G. Gage, *Proc. R. Soc. London Ser. B* **265**, 97 (1998).
34. P. E. Eady, *J. Zool. (London)* **253**, 47 (2001).
35. S. Pitnick, G. T. Miller, J. Reagan, B. Holland, *Proc. R. Soc. London Ser. B* **268**, 1071 (2001).
36. We thank J. Reagan and D. Trinkaus for technical assistance and J. Alcock, T. R. Birkhead, A. Bjork, W. D. Brown, T. L. Karr, L. A. McGraw, M. Polak, R. R. Snook, W. T. Starmer, and L. L. Wolf for comments on an earlier draft of the manuscript. Supported by NSF grants DEB-9806649 and DEB-0075307 (S.P.).

Ecological Predictions and Risk Assessment for Alien Fishes in North America

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Methods of risk assessment for alien species, especially for nonagricultural systems, are largely qualitative. Using a generalizable risk assessment approach and statistical models of fish introductions into the Great Lakes, North America, we developed a quantitative approach to target prevention efforts on species most likely to cause damage. Models correctly categorized established, quickly spreading, and nuisance fishes with 87 to 94% accuracy. We then identified fishes that pose a high risk to the Great Lakes if introduced from unintentional (ballast water) or intentional pathways (sport, pet, bait, and aquaculture industries).

Increased trade and tourism associated with globalization have facilitated one of the least reversible human-induced global changes now under way: the homogenization of Earth's biota through the establishment and spread of alien species (1, 2). Given the myriad detrimental impacts attributed to alien species in invaded ecosystems (3, 4) and the limited possibilities for eradication, predict-

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ing potential alien species and preventing their establishment are important policy goals (5). Invasion biology has, however, been plagued by a paradox that has hindered prevention. On the one hand, there is a widespread perception that diagnostic characteristics of weedy species have long since been identified (6). Current risk-screening protocols, such as the Weed Risk Assessment of Australia (7) and the Ecological Risk Assessment Framework of the U.S. Government (8), are based on largely qualitative categorizations of such putative diagnostic characteristics. On the other hand, there is a widespread perception that predictions about which species will invade are impossible (9). This perception has emerged from searching for