Y1102. The probability of bursting was not affected by Y1102. Instead, Y1102 increased the probability that a channel will open in the burst mode by increasing the energetic favorability of the activation transition.

We next computed action potentials using simulated S1102 or Y1102 channels. The subtle changes in gating did not alter action potentials (Fig. 3). However, when we simulated a concentration-dependent block of the rapidly activating delayed rectifier potassium currents (I_{Kr}), a common side effect of many medications and hypokalemia, our computations predicted that Y1102 would induce action potential prolongation and early afterdepolarizations (EADs). EADs are a cellular trigger for ventricular tachycardia (*15*). Thus, computational analyses indicated that Y1102 increased the likelihood of QT prolongation, EADs, and arrhythmia in response to drugs (or drugs coupled with hypokalemia) (fig. S2) that inhibit cardiac repolarization.

We conclude that Y1102, a common SCN5A variant in Africans and African Americans, causes a small but inherent and chronic risk of acquired arrhythmia. The key to therapy is prevention. The identification of a common variant that causes a subtle increase in the risk of life-threatening arrhythmias will facilitate prevention through rapid identification of populations at risk. We estimate that 4.6 million African Americans carry Y1102 (*16*). Most of these individuals will never have an arrhythmia because the effect of Y1102 is subtle. However, in the setting of additional acquired risk factors, particularly common factors such as medications, hypokalemia, or structural heart disease, these individuals are at increased risk. Successful strategies for prevention, including avoidance of certain medications (*17–19*), maintenance of a normal serum potassium concentration (*20*), and beta-blocker therapy (*21*), are available. Additional, longitudinal studies will be required to confirm the predictive utility of Y1102.

References and Notes

1. Z. J. Zheng, J. B. Croft, W. H. Giles, G. A. Mensah, *Circulation* **104**, 2158 (2001).
2. D. M. Roden, *Cardiovasc. Res.* **50**, 224 (2001).
3. M. E. Gellens *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 554 (1992).
4. Q. Wang *et al.*, *Cell* **80**, 805 (1995).
5. P. B. Bennett, K. Yazawa, N. Makita, A. L. George Jr., *Nature* **376**, 683 (1995).
6. Q. Chen *et al.*, *Nature* **392**, 293 (1998).
7. J. J. Schott *et al.*, *Nature Genet.* **23**, 20 (1999).
8. H. L. Tan *et al.*, *Nature* **409**, 1043 (2001).
9. M. T. Keating, M. C. Sanguinetti, *Cell* **104**, 569 (2001).
10. See supplementary material on *Science* Online.
11. R. H. An *et al.*, *Circ. Res.* **83**, 141 (1998).
12. O. P. Hamill, A. Marty, E. Neher, B. Sakmann, F. J. Sigworth, *Pflugers Arch.* **391**, 85 (1981).
13. C. E. Clancy, Y. Rudy, *Nature* **400**, 566 (1999).
14. _____, *Circulation* **105**, 1208 (2002).
15. D. M. Roden, *Eur. Heart. J.* **14** (suppl H), 56 (1993).
16. U.S. Census Bureau data are available at www.census.gov/main/www/cen2000.html.
17. Y. G. Yap, J. Camm, *Br. Med. J.* **320**, 1158 (2000).
18. M. J. Kilborn, R. L. Woosley, *Br. Med. J.* **322**, 672 (2001).
19. Information on drugs that prolong the QT interval and/or induce torsade de pointes can be found at www.torsades.org.
20. S. J. Compton *et al.*, *Circulation* **94**, 1018 (1996).
21. A. J. Moss *et al.*, *Circulation* **101**, 616 (2000).

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Supporting Online Material

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Mechanisms of Adaptation in a Predator-Prey Arms Race: TTX-Resistant Sodium Channels

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Populations of the garter snake *Thamnophis sirtalis* have evolved geographically variable resistance to tetrodotoxin (TTX) in a coevolutionary arms race with their toxic prey, newts of the genus *Taricha*. Here, we identify a physiological mechanism, the expression of TTX-resistant sodium channels in skeletal muscle, responsible for adaptive diversification in whole-animal resistance. Both individual and population differences in the ability of skeletal muscle fibers to function in the presence of TTX correlate closely with whole-animal measures of TTX resistance. Demonstration of individual variation in an essential physiological function responsible for the adaptive differences among populations is a step toward linking the selective consequences of coevolutionary interactions to geographic and phylogenetic patterns of diversity.

Complex phenotypes such as performance or resistance typically comprise physiological, morphological, and behavioral components (*1*). Although selection acts directly on complex phenotypes themselves (*2, 3*), it is ultimately the evolution of these underlying components that shapes patterns of adaptation among taxa. The indirect evolutionary response of physiological mechanisms and other causal factors requires individual variation in physiology that is correlated with a performance measure that is in turn correlated with individual differences in fitness. This cascade of effects links physiology to fitness and, given appropriate genetic variation in physiology, is thought to lead to adaptive diversification in performance and the underlying factors that influence it (*3*). Considerable effort in integrative biology has been devoted to understanding the mechanistic basis of complex traits that differ among populations or species (*4*). However, few studies have successfully linked presumably adaptive differences in physiological function among populations to the analogous individual and genetic variation within populations that is required for natural selection to explain adaptive diversification in physiological traits.

Coevolutionary interactions generate particularly rapid and complex patterns of adaptive diversification among populations (*5*); theoretical developments emphasize the critical role that geographic variation in selection plays in determining the dynamic of coevolution and predict that coevolutionary outcomes will vary markedly among populations of the same species (*5–7*). Coevolutionary interactions therefore are particularly suitable for studying the link between mechanisms and patterns of adaptation. We investigated a performance trait, tetrodotoxin (TTX) resistance, at the phenotypic interface of the "arms race" between a garter snake predator, *Thamnophis sirtalis*, and its toxic prey, newts of the genus *Taricha*.

The skin of *Taricha granulosa* contains TTX, a potent neurotoxin that blocks voltage-gated sodium channels in nerve and muscle tissue, thereby inhibiting the propagation of action potentials (APs) and paralyzing nerve and muscle function (*8, 9*). TTX binds to sodium channels in many different tissues, but death from TTX intoxication usually results from respiratory failure (*10, 11*). Some

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populations of newts of the genus *Taricha* possess extremely high quantities of TTX that provide an effective defense against predators (*11–13*). The only predator known to be resistant to this toxin is the common garter snake *T. sirtalis* (*14, 15*).

Variation in TTX resistance among populations of *T. sirtalis* is extreme, spanning three orders of magnitude (*16*). Historical, geographic, and intrapopulational evidence suggests that this variation has resulted from a coevolutionary arms race with toxic newts. Effects of attacking a toxic newt range from reduced locomotor performance to paralysis and even death, depending on the quantitative level of resistance of the individual snake (*14, 15*). Within populations, whole-animal resistance to TTX varies among individual snakes, is nonplastic, and has a heritable basis (*15, 17*). Genetically based physiological tradeoffs between the level of TTX resistance and locomotor performance are evident in all populations tested; more-resistant snakes have slower maximum crawl speeds (*18*). Among populations, the levels of both newt toxicity and garter snake resistance are variable but tightly matched (*14–16, 19, 20*). Phylogeographic evidence indicates that TTX resistance has evolved independently at least twice within *T. sirtalis* in western North America (*16, 21*).

We examined the physiological mechanisms underlying whole-animal TTX resistance in four populations of garter snakes (*22*). These populations represent two independent evolutionary origins of TTX resistance and span the range of variation in resistance observed across all populations in western North America (*16, 21*) (Fig. 1). Because genetically based differences in sodium channels are known to alter sensitivity to TTX in some taxa, including some species of *Taricha* and pufferfish that express TTX-resistant sodium channels in skeletal muscle or peripheral nerve tissue (*10, 23–25*), we investigated this potential mechanism of TTX resistance in *T. sirtalis.*

We measured skeletal muscle resistance by assessing the ability of muscle fibers to propagate APs in various concentrations of TTX (*22*). The propagation of APs was blocked at different concentrations of TTX in the four populations. Differences in the TTX concentration required to block APs matched the pattern of whole-animal resistance among populations. Skeletal muscle fibers of snakes from the most resistant population (Willow Creek, California) conducted APs in the presence of 100 times the concentration of TTX (1.0×10^{-5} M) that blocked AP propagation in four of six snakes from the least resistant population (Bear Lake, Idaho; 1.0×10^{-7} M TTX). AP propagation in skeletal muscle fibers of snakes from the two intermediate resistance populations was blocked at TTX concentrations between 5.0×10^{-7} M and 17.5×10^{-7} M. Because of the specificity with which TTX binds to and blocks sodium channels, one component of the differences in action potential propagation among populations is sodium channel sensitivity to TTX.

We used a reduction in the rise rate of APs as an estimator of the level of TTX block in the sodium channels expressed in skeletal muscle fibers (*22*). AP rise rates of snakes from the three lower resistance populations were slowed in TTX concentrations (under 1.0×10^{-6} M) that had little effect on the APs of snakes from the most resistant population, Willow Creek (Fig. 2). Furthermore, AP rise rates recorded from the Bear Lake population were severely reduced compared to Warrenton and Benton, Oregon, at low TTX concentrations (3.0×10^{-8} M to 1.0×10^{-7} M). To quantify differences in sodium channel TTX resistance, we compared the AP rise rate ratios (*22*) of individual snakes over the range of TTX concentrations (Fig. 3A). Significant differences among populations in the relationship between AP rise rate ratio and TTX concentration were observed ($\delta = 0.847$, $P < 0.001$), including significant differences among each pair of populations (Bear Lake versus Warrenton: $\delta = 1.11$, $P = 0.003$; Bear Lake versus Benton: $\delta = 1.156$, $P = 0.0006$; Bear Lake versus Willow Creek: $\delta = 0.913$, $P = 0.001$; Warrenton versus Willow Creek: $\delta = 0.805$, $P = 0.001$; Benton versus Willow Creek: $\delta = 1.039$, $P = 0.002$) except Benton and Warrenton (for which $\delta = 1.422$, $P = 0.061$). These results suggest that snakes from different populations express sodium channels in their skeletal muscle fibers with differential binding affinities for TTX.

Estimates of the TTX concentration required to block 50% of the sodium channels, expressed as K_d (*22*), in skeletal muscle fibers of a given population illustrate large differences among the four populations (Fig. 3B). Moreover, these differences correspond closely to those in the amount of TTX re-

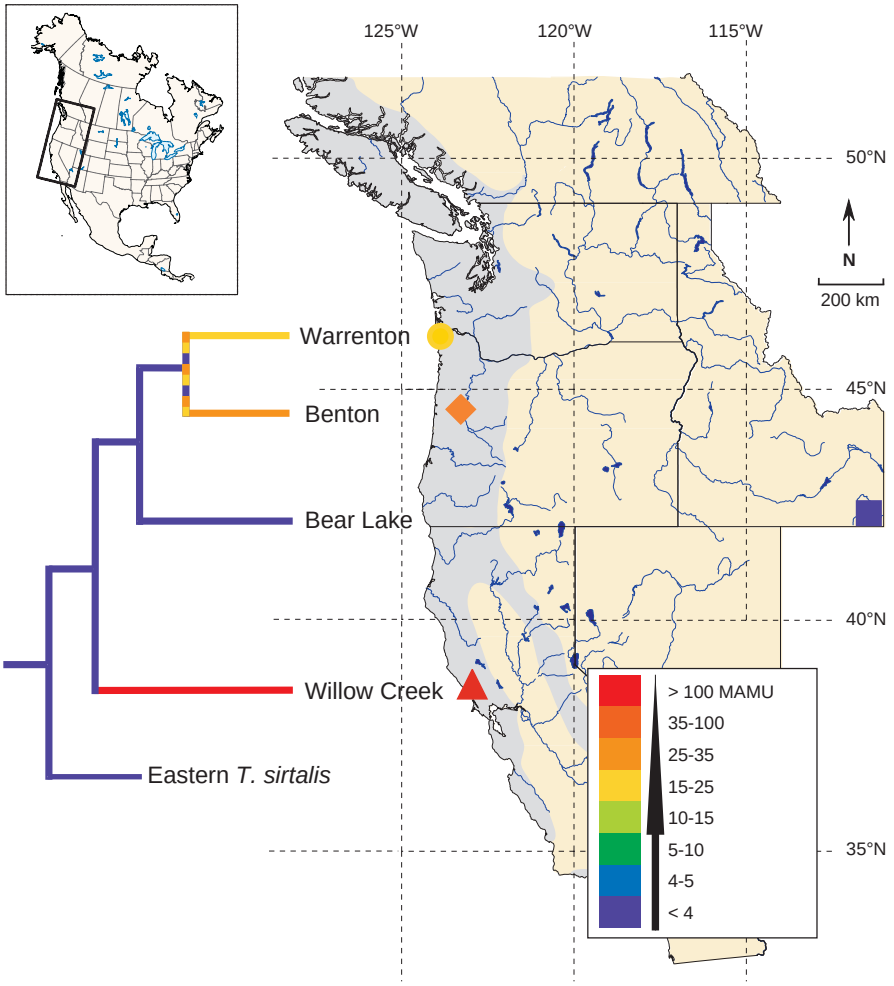

Fig. 1. Localities and relationships of *Thamnophis sirtalis* in this study. Gray shaded region indicates range of newts of the genus *Taricha*. Colors correspond to level of whole-animal TTX resistance; legend includes all levels of resistance observed throughout western North America (*16*). Phylogeographic relationships and resistance evolution for these four populations are based on mitochondrial DNA analysis of 19 western populations of *T. sirtalis* (*16, 21*). MAMU, mass adjusted mouse units.

quired to reduce whole-animal performance. Estimated 95% confidence intervals (CIs) for both measures are nonoverlapping, and linear regression of whole-animal TTX resistance on sodium channel resistance shows a clear quantitative relationship between these measures across populations (Fig. 3B). The estimated K_d for the Bear Lake population (K_d = 3.3×10^{-8} M TTX; 95% CI: 2.19×10^{-8} to 4.41×10^{-8} M TTX), which does not occur with newts, is of the same order as that previously reported (1.0×10^{-8} M TTX) for sodium channels expressed in the skeletal muscle of *T. sirtalis* from unknown localities (*10, 26–28*). The estimated K_d value for the Willow Creek population (4.2×10^{-5} M TTX; 95% CI: 5.5×10^{-6} to 7.9×10^{-5} M TTX) is comparable to K_d values estimated for skeletal muscle sodium channels found in six species of tetrodontoid fish ($K_d > 3.0 \times 10^{-6}$ M TTX) (*24*). The binding affinity estimates for sodium channels in skeletal muscle fibers suggest that snakes from the most TTX-resistant population (Willow Creek) express highly TTX-resistant sodium channels and that snakes from populations with intermediate resistance express sodium channels with TTX resistance levels between those found in snakes from Willow Creek and Bear Lake.

To determine whether skeletal muscle resistance was an important component of individual differences in whole-animal resistance, we tested whether skeletal muscle resistance in the two intermediate-resistance populations predicted individual differences in whole-animal TTX resistance. A general linear model (*22*) detected significant, positive regressions of whole-animal TTX resistance on skeletal muscle resistance to TTX within each population (Fig. 4). Because of large variation in TTX resistance, only three

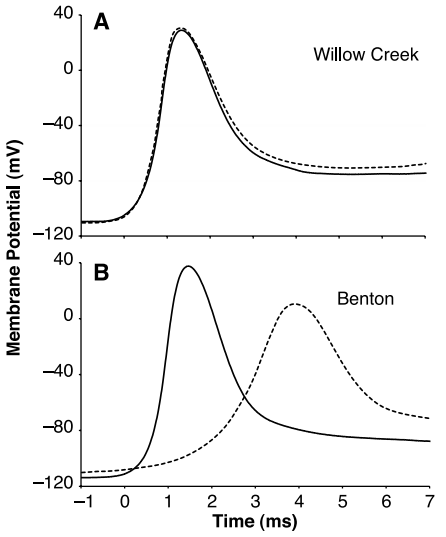

Fig. 2. Intracellular action potentials recorded in snake skeletal muscle fibers. The same TTX concentration (5.0×10^{-7} M) has different effects on the AP rise rates of snakes from different populations. In the absence of TTX (solid lines), the AP rise rate and the maximum membrane voltage reached during an action potential are similar for the action potentials recorded in the skeletal muscle fibers of snakes from (**A**) Willow Creek (maximum rise rate, 235 mV/ms; $V_m$max, 29.0 mV; resting V_m, −109 mV) and (**B**) Benton (maximum rise rate, 255 mV/ms; $V_m$max, 37.6 mV; resting V_m, −113 mV). At concentrations of 5.0×10^{-7} M TTX (dashed lines), these parameters are reduced for the AP recorded in the snake from Benton County, OR (maximum rise rate, 90 mV/ms; $V_m$max, 10.5 mV; resting V_m, −110 mV), but are unaffected in the snake from Willow Creek, CA (maximum rise rate, 235 mV/ms; $V_m$max, 30.7 mV; resting V_m, −110 mV).

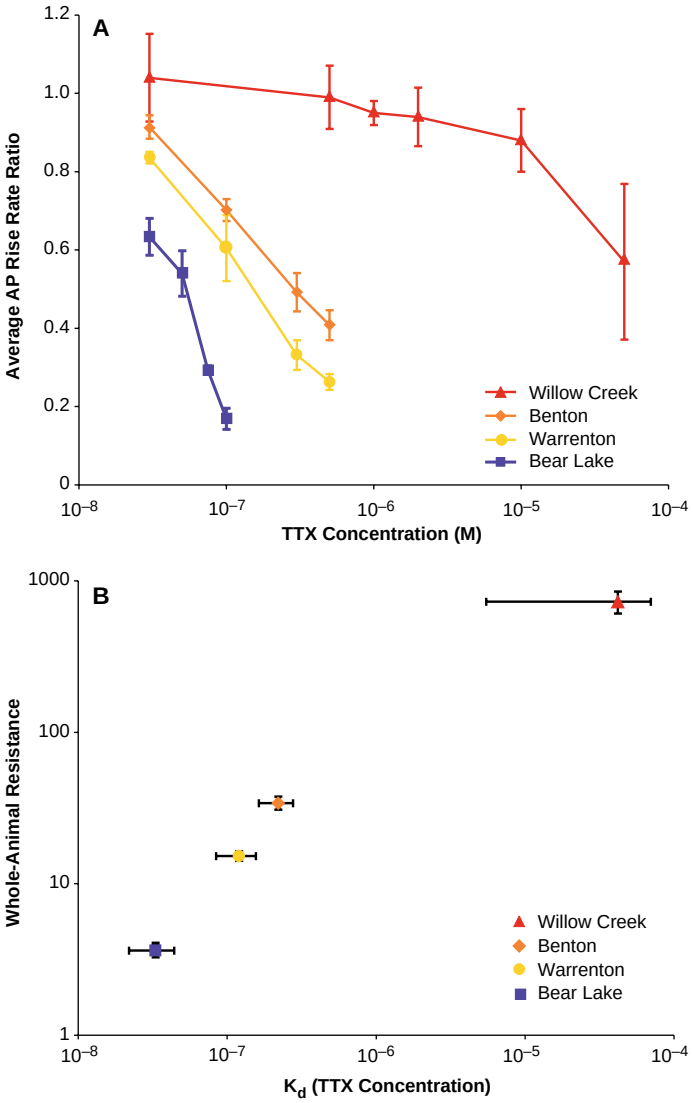

Fig. 3. Population differences in whole-animal and sodium channel TTX resistance for four populations. (**A**) The maximum rise rate ratio of action potentials as a function of TTX concentration. Means (±SE) for all snakes that fired APs in each population are shown at each concentration tested. (**B**) The among-population relationship between whole-animal TTX resistance (measured as the TTX dose in mass adjusted mouse units that produces 50% reduction in crawl speed) and sodium channel TTX resistance (measured as K_d, the TTX molar concentration required to block 50% of sodium channels). Linear regression indicates a significant relationship between these measures at the population level [$F(1,3) = 2001.5$, $R^2 = 0.999$, $P = 0.0005$]. Colors correspond to level of whole-animal resistance as in Fig. 1.

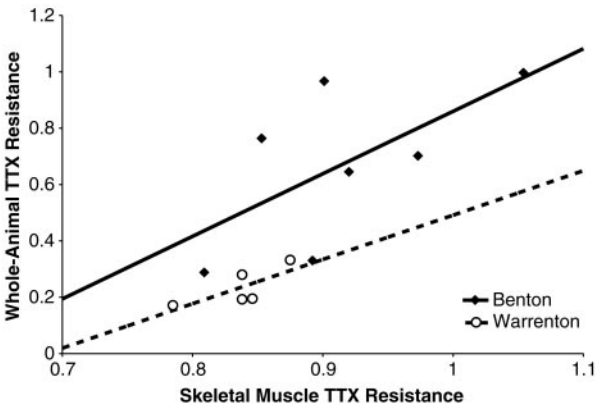

Fig. 4. Individual variation in whole-animal TTX resistance is predicted by skeletal muscle TTX resistance in two populations of *T. sirtalis* [Benton: $y = -1.3602 + 2.2203x$, $R^2 = 0.41$, $n = 7$; Warrenton: $y = -1.0817 + 1.5731x$, $R^2 = 0.55$, $n = 5$; $F(1,8) = 6.82$, $P = 0.03$ for overall model] (*22*).

individuals from Willow Creek could be tested at common doses (they exhibited a similar but nonsignificant positive relationship); individual whole-animal resistance measures were not available for Bear Lake individuals. The concordance between whole-animal and skeletal muscle TTX resistance indicates that the physiological mechanisms that allow skeletal muscle fibers to function in increased concentrations of TTX are at least one component of individual differences in the effects of TTX on locomotor performance. Unexplained variance suggests that additional underlying mechanisms of whole-animal TTX resistance remain to be identified. Such mechanisms may include the ability of other nerve and muscle tissues to function in TTX.

The coevolutionary arms race between *T. sirtalis* and newts defended by TTX has led to remarkable adaptive divergence in whole-animal TTX resistance among snake populations. This divergence includes independent evolution of resistance in at least two distinct lineages of garter snakes, as well as substantial quantitative variation in level of TTX resistance among populations (*16*). Some of this variation may result in part from observed trade-offs between resistance and other physiological functions (*16, 18*). The results we present here demonstrate one physiological mechanism underlying the adaptive differences among populations, but just as important, they show that variation in the same physiological function exists among individuals within populations. Selection resulting from the interactions of individual predators and prey may therefore act indirectly on this fundamental physiological function. Although we do not know that these individual differences in skeletal muscle resistance are genetically based, two points suggest that the potential exists for this physiological variation to respond to selection. First, whole-animal TTX resistance is known to be highly heritable in garter snakes (*15*), which suggests that heritable variation is also present in the mechanisms that underlie it. Second, the TTX sensitivity of skeletal muscle sodium channels in other vertebrates is affected by genetic changes that alter amino acid sequences (*25, 29, 30*). At this point, it is unclear whether the parallel evolution of TTX resistance in separate lineages of *T. sirtalis* has occurred through identical or unique genetic mechanisms, but the genes that encode sodium channels expressed in the skeletal muscle fibers of resistant snakes appear to be good candidates with which to explore this question.

References and Notes

1. S. J. Arnold, *Am. Zool.* **23**, 347 (1983).
2. T. Garland Jr., A. F. Bennett, C. B. Daniels, *Experientia* **46**, 530 (1990).
3. T. J. Garland, P. A. Carter, *Annu. Rev. Physiol.* **56**, 579 (1994).
4. T. J. Garland, S. C. Adolph, *Annu. Rev. Ecol. Syst.* **22**, 193 (1991).
5. J. N. Thompson, *The Coevolutionary Process* (Univ. of Chicago Press, Chicago, 1994).
6. S. L. Nuismer, J. N. Thompson, R. Gomulkiewicz, *Proc. R. Soc. London Ser. B* **266**, 605 (1999).
7. J. N. Thompson, *Am. Nat.* **153**, S92 (1999).
8. G. M. Lipkind, H. A. Fozzard, *Biophys. J.* **66**, 1 (1994).
9. T. Narahashi, J. W. Moore, R. N. Poston, *Science* **156**, 976 (1967).
10. A. L. Goldin, *Annu. Rev. Physiol.* **63**, 871 (2001).
11. E. D. Brodie Jr., *Copeia* **1968**, 307 (1968).
12. _____, J. L. Hensel Jr., J. A. Johnson, *Copeia* **1974**, 506 (1974).
13. H. S. Mosher, F. A. Fuhrman, H. D. Buchwald, H. G. Fischer, *Science* **144**, 1100 (1964).
14. E. D. Brodie III, E. D. Brodie Jr., *Bioscience* **49**, 557 (1999).
15. _____, *Evolution* **44**, 651 (1990).
16. E. D. Brodie Jr., B. J. Ridenhour, E. D. Brodie III, *Evolution*, in press.
17. B. J. Ridenhour, E. D. Brodie Jr., E. D. Brodie III, *Copeia* **1999**, 531 (1999).
18. E. D. Brodie III, E. D. Brodie Jr., *Evolution* **53**, 626 (1999).
19. _____, *Evolution* **45**, 221 (1991).
20. C. T. Hanifin, M. Yotsu-Yamashita, T. Yasumoto, E. D. Brodie III, E. D. Brodie Jr., *J. Chem. Ecol.* **25**, 2161 (1999).
21. F. J. Janzen, J. G. Krenz, T. S. Haselkorn, E. D. Brodie Jr., E. D. Brodie III, *Mol. Ecol.*, in press.
22. See supporting data on *Science* Online.
23. C. Y. Kao, F. A. Fuhrman, *Toxicon* **5**, 24 (1967).
24. Y. Kidokoro, A. D. Grinnell, D. C. Eaton, *J. Comp. Physiol.* **89**, 59 (1974).
25. M. Yotsu-Yamashita *et al.*, *Biochem. Biophys. Res. Commun.* **267**, 403 (2000).
26. J. L. Penzotti, H. A. Fozzard, G. M. Lipkind, S. C. Dudley, *Biophys. J.* **75**, 2647 (1998).
27. J. H. Caldwell, R. L. Milton, *J. Physiol.* **401**, 145 (1988).
28. W. M. Roberts, *J. Physiol.* **388**, 213 (1987).
29. J. H. Caldwell, D. T. Campbell, K. G. Beam, *J. Gen. Physiol.* **87**, 907 (1986).
30. H. Terlau *et al.*, *FEBS Lett.* **293**, 93 (1991).

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Sexual Selection, Temperature, and the Lion's Mane

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The mane of the African lion (*Panthera leo*) is a highly variable trait that reflects male condition and ambient temperature. We examined the consequences of this variation in a long-term study of lions in the Serengeti National Park, Tanzania. Mane darkness indicates nutrition and testosterone and influences both female choice and male-male competition. Mane length signals fighting success and only appears to influence male-male assessment. Dark-maned males enjoy longer reproductive life-spans and higher offspring survival, but they suffer higher surface temperatures, abnormal sperm, and lower food intake during hot months of the year. Maned males are hotter than females, and males have lighter and/or shorter manes in hotter seasons, years, and habitats. This phenotypic plasticity suggests that the mane will respond to forecasted increases in ambient temperature.

Sexually selected indicator traits reflect male health and vigor (*1*), revealing how well individuals withstand environmental stress (*2*, *3*). Environmental effects on trait morphology can be substantial (*4*, *5*), outweighing both genetic effects (*6*) and reproductive advantages (*7*). Changing environmental conditions can alter trait costs, leading to the evolutionary loss of sexual ornaments (*8*) or possibly even to species extinction (*9*). The global environment is undergoing rapid change due to anthropogenic disturbance (*10*), and these changes have already altered some sexually selected behavior (*11*, *12*). Sexually selected morphological traits may also be vulnerable, depending on the relative importance of ecological effects, reproductive benefits, and a trait's phenotypic and/or genetic plasticity, as well as the magnitude of environmental change.

Here we examine the evolutionary and ecological factors influencing the mane of the African lion. Manes are sexually dimorphic, develop at puberty, and are highly variable; thus, the mane has long been considered a sexually selected trait (*13*). Mane size and darkness are reduced in populations and subspecies living in hot climates [see supporting

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