Low Potential for Climatic Stress Adaptation in a Rainforest *Drosophila* Species

A. A. Hoffmann,* R. J. Hallas, J. A. Dean, M. Schiffer

The ability of sensitive rainforest species to evolve in response to climate change is largely unknown. We show that the Australian tropical rainforest fly *Drosophila birchii* exhibits clinal variation in desiccation resistance, but the most resistant population lacks the ability to evolve further resistance even after intense selection for over 30 generations. Parent-offspring comparisons indicate low heritable variation for this trait but high levels of genetic variation for morphology. *D. birchii* also exhibits abundant genetic variation at microsatellite loci. The low potential for resistance evolution highlights the importance of assessing evolutionary potential in targeted ecological traits and species from threatened habitats.

Many species are currently experiencing novel conditions because of habitat fragmentation and climate change, resulting in rapid shifts in species distributions as well as genetic changes in quantitative traits (*1*–*3*). It is often assumed that populations have abundant genetic variation in quantitative traits for adaptation (*4*), but this is on the basis of studies of genetic variation in generalist model species with broad distributions.

Centre for Environmental Stress and Adaptation Research, La Trobe University, Bundoora, Victoria 3086, Australia.

*To whom correspondence should be addressed. E-mail: A.Hoffmann@latrobe.edu.au

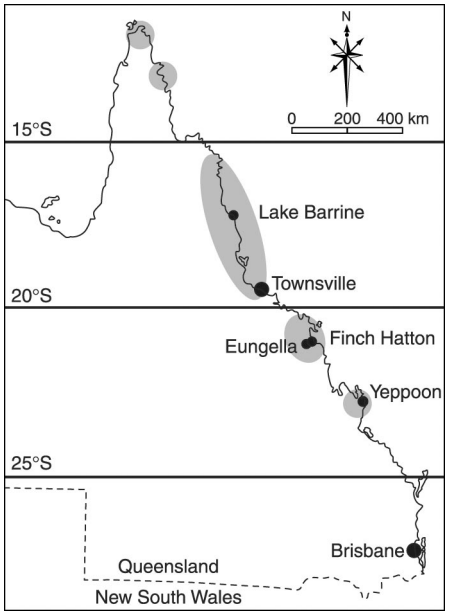

Fig. 1. Regions of northeastern Australia where *D. birchii* are found (shaded areas). The locations of the four sites sampled repeatedly to test for laboratory adaptation (small circles) and two major cities (medium circles) are also indicated.

Desiccation resistance is associated with the distribution of many species of insects. Species restricted to tropics, particularly rainforests, have low levels of resistance, reflecting the continuous high humidity encountered in these habitats (*5*). As conditions become drier and as fragmentation generates edge effects that alter the microclimate within fragments, desiccation stress experienced in rainforests is likely to increase (*6*). Desiccation resistance has a high heritability in populations of widespread *Drosophila* species, reflecting a high evolutionary potential and allowing populations with increased levels of resistance to evolve rapidly (*7*–*10*). However, these studies have been undertaken on a restricted set of generalist species that are relatively widespread and easily cultured under artificial conditions, and their relevance to species from restricted humid habitats remains to be assessed.

Drosophila birchii, which is restricted to rainforests of northern Australia and New Guinea (*11*), is sensitive to desiccation, unlike its closely related sibling species, *D. serrata*, that has a broader distribution (*12*, *13*). In northern Queensland, *D. birchii* is normally collected only from rainforest pockets, including a region near Yeppoon where an isolated population marks the southern distribution limit of *D. birchii* (Fig. 1).

To examine geographic variation in desiccation resistance in *D. birchii*, we characterized isofemale lines from 12 sites, including Yeppoon, along the eastern coast for resistance (*14*). There was a weak clinal pattern of resistance increasing with higher latitudes (Fig. 2), in agreement with results from several other *Drosophila* species (*15*, *16*). This geographic pattern is likely to reflect climatic selection because of reducing levels of precipitation with higher latitudes, which is related to water-loss rates in insects (*17*). Climatic averages obtained from the Australian Bureau of Meteorology weather stations near the collection sites north of Townsville (latitude 19.3°S) indicate a mean annual rainfall exceeding 2000 mm and more than 120 rain days per year, compared to a rainfall below 1500 mm and less than 100 rain days per year at sites south of Townsville.

To test the ability of *D. birchii* to respond to selection for increased desiccation resistance, we exposed flies to a desiccation stress until 80 to 90% had died and then bred from the survivors. This process was repeated for 30 cycles over 50 culture generations (*14*). The Finch Hatton population was used for these experiments because it had a high level of resistance in the geographic comparison. Selection failed to increase resistance substantially (Fig. 3), suggesting a lack of genetic variation for increased resistance that is contrary to expectations and findings from other *Drosophila* species selected with the use of similar methods (*7*–*10*). Comparisons of the lengths of time taken for 50% of flies to die (LT50s, estimated by linear interpolation) indicate no evidence for a significant selection response in a direct comparison of all selected and control lines after 28 cycles of selection [nested analysis of variance (ANOVA), $F_{(1,4)} = 1.92$, $P = 0.24$]. Two of the selected lines may have exhibited slightly higher resistance (Fig. 3), but differences between the two sets of lines were small (11.6% on average) as compared to increases of two- to fivefold in *D. melanogaster*, *D.*

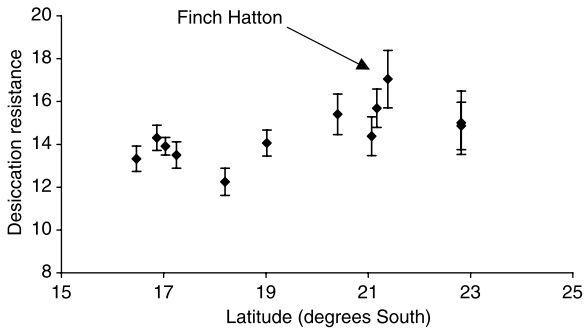

Fig. 2. Clinal variation in desiccation resistance (hours to 50% mortality) in *D. birchii*. Points represent the means of isofemale lines from 12 locations. Error bars are standard errors based on isofemale line means. A regression of desiccation resistance onto latitude based on isofemale line means is significant [$F_{(1,67)} = 11.20$, $P = 0.001$, $r^2 = 0.14$], as is the equivalent regression based on location means [$F_{(1,10)} = 7.61$, $P = 0.02$, $r^2 = 0.43$], reflecting an increase in resistance at higher latitudes. The Finch Hatton population used for selection is indicated.

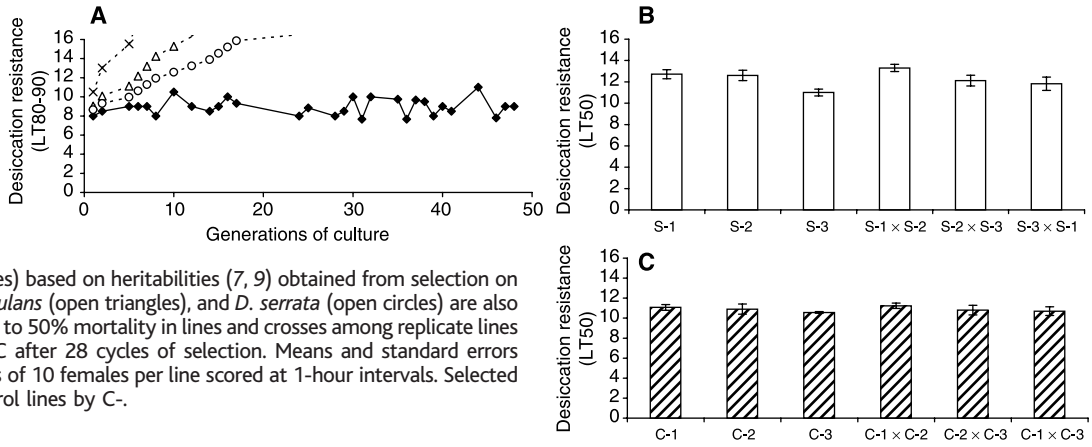

Fig. 3. Desiccation resistance of lines during selection and results of replicate line crosses. (**A**) Desiccation resistance scored at 24° to 26°C averaged across lines as hours taken to 80 to 90% mortality (solid line). There were 30 cycles of selection (solid diamonds) over 50 culture generations. The anticipated responses to selection (hatched lines) based on heritabilities (*7*, *9*) obtained from selection on *D. melanogaster* (crosses), *D. simulans* (open triangles), and *D. serrata* (open circles) are also presented. (**B** and **C**) Time (hours) to 50% mortality in lines and crosses among replicate lines tested at the same time at 25°C after 28 cycles of selection. Means and standard errors (bars) are based on five replicates of 10 females per line scored at 1-hour intervals. Selected lines are represented by S-; control lines by C-.

simulans, and *D. serrata* [from data in (*7*, *8*, *10*)]. Comparisons of the 10% and 90% mortality points also indicated that the response to selection was not significant.

Selection responses can be confounded by several factors, including inbreeding and laboratory adaptation (*18*, *19*). The independent replicate lines were crossed to ensure that the absence of a selection response was not a consequence of inbreeding depression influencing desiccation resistance (*14*). Because flies derived from crosses between lines had a similar level of resistance to those derived from crosses within lines (Fig. 3), there was no evidence that inbreeding effects countered any selection response. Further selection was also attempted on synthetic populations that had been established by combining flies from the independently maintained replicate lines in an attempt to increase genetic variation available for selection. However, a further six generations of selection on these synthetic populations failed to increase desiccation resistance. Because neither inbreeding nor limited genetic variation within replicate lines appeared to have influenced the selection response, genes increasing desiccation resistance do not appear to be segregating in the Finch Hatton population.

We carried out parent-offspring comparisons to directly measure heritable variation for desiccation resistance in a new mass-bred population established from flies collected at Finch Hatton (*14*). This population contained abundant genetic variation; an analysis of variation at five microsatellite loci for 60 females indicated an average heterozygosity level (H) of 0.65 ± 0.03 and an average number of alleles per locus (A) of 8.4 ± 2.4. These are similar to values obtained for H (0.56 ± 0.06) and A (7.4 ± 1.4) from field Finch Hatton *D. birchii* and are also comparable to H and A in widespread species, including *D. melanogaster* (*20*). Narrow-sense heritability estimates for desiccation resistance based on single-parent or midparent comparisons were zero (Table 1), reflecting that there was little resemblance between parents and their offspring for this trait and that there was a low level of heritable variation for desiccation resistance in this population. By contrast, heritabilities for wing size and wing aspect in the same population were intermediate to high (Table 1) and consistent with heritability estimates for morphological traits in widespread *Drosophila* species (*21*, *22*).

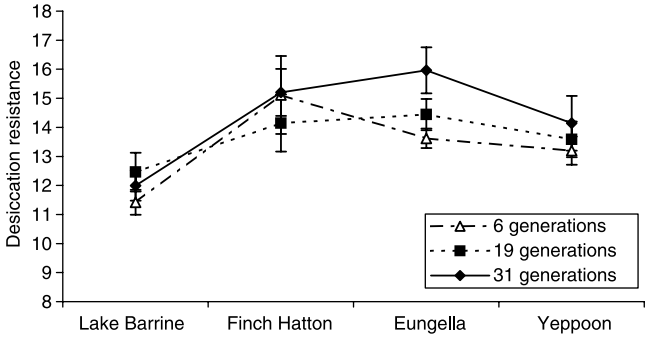

Fig. 4. Effects of laboratory adaptation on desiccation resistance (hours to 50% mortality). Lines were collected from four sites on each of three occasions and held in culture for 6, 19, or 31 generations before testing. Means and standard errors (bars) are on the basis of means of isofemale lines. For locations, see Fig. 1.

Table 1. Parent-offspring comparisons for desiccation resistance and wing traits in *D. birchii* from the Finch Hatton population.

Parental comparison	Number of families	Regression coefficient	Probability	Narrow-sense heritability	Standard error of heritability
		Desiccation resistance			
Female	122	<0.001	0.992	0	0.090
Male	121	−0.058	0.453	0	0.154
Midparent	113	−0.037	0.64	0	0.095
		Wing size (centroid)			
Female	66	0.353	0.003	0.706	0.230
Male	66	0.193	0.054	0.386	0.108
		Wing aspect			
Female	66	0.411	<0.001	0.821	0.198
Male	66	0.340	<0.001	0.680	0.158

Because laboratory adaptation can influence desiccation resistance as well as other traits in *Drosophila* (*23*, *24*) and confound selection responses, we tested for changes in resistance in lines maintained in the laboratory for different lengths of time. Isofemale lines were established from field flies collected from four sites in 2000 as well as from the same sites in 2001 and 2002. These lines were evaluated simultaneously for desiccation resistance in 2002 (*14*). Lines from the most northern population were consistently less resistant than those from the southern populations (Fig. 4), suggesting stable population differences despite varying lengths of time in laboratory culture. An ANOVA indicated significant differences among populations [$F_{(3,65)} = 9.07$, $P < 0.001$] but no effect of collection year [$F_{(2,65)} = 1.84$, $P = 0.167$] or interaction between year and population [$F_{(6,65)} = 0.71$, $P = 0.639$]. The population differences match those evident from the more extensive clinal collection, including a slight reduction in resistance in Yeppoon as compared to Finch Hatton (compare in Fig. 3). Laboratory culturing, therefore, did not influence the desiccation resistance of *D. birchii* and confound the selection results.

Differences in desiccation resistance among *D. birchii* populations suggest that there has

been a past history of selection on this trait. Yet, low levels of genetic variation for desiccation resistance appear to be preventing any further increases in resistance in this rainforest species despite ample genetic variation in other traits and at neutral markers as evident from the microsatellite results. Our results show that genetic variation in neutral markers can provide an incomplete picture of the evolutionary potential of populations, consistent with the weak association between genetic diversity as measured by quantitative methods and that measured by molecular methods (*25*). The absence of a selection response for traits linked to climatic stress in this study and in a few other cases (*26*) suggests that levels of variation must be evaluated for ecologically relevant traits in those species that are threatened by climate change and fragmentation, including endangered species (*27*).

References and Notes

1. W. E. Bradshaw, C. M. Holzapfel, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 14509 (2001).
2. L. Hughes, *Trends Ecol. Evol.* **15**, 56 (2000).
3. M. S. Warren *et al.*, *Nature* **400**, 65 (2001).
4. R. C. Lewontin, *The Genetic Basis of Evolutionary Change* (Columbia Univ. Press, New York, 1974).
5. P. A. Parsons, *Evol. Biol.* **14**, 297 (1982).
6. T. E. Lovejoy *et al.*, in *Conservation Biology: The Science of Scarcity and Diversity*, M. E. Soule, Ed. (Sinauer Associates, Sunderland, MA, 1986), pp. 257–285.
7. A. A. Hoffmann, P. A. Parsons, *Biol. J. Linn. Soc.* **37**, 117 (1989).
8. M. W. Blows, A. A. Hoffmann, *Evolution* **47**, 1255 (1993).
9. G. Gibbs, A. K. Chippindale, M. R. Rose, *J. Exp. Biol.* **200**, 1821 (1997).
10. A. A. Hoffmann, P. A. Parsons, *J. Evol. Biol.* **6**, 643 (1993).
11. F. J. Ayala, *Evolution* **19**, 538 (1965).
12. M. J. Hercus, A. A. Hoffmann, *Genetics* **151**, 1493 (1999).
13. N. L. Jenkins, A. A. Hoffmann, *Aust. J. Entomol.* **40**, 41 (2001).
14. Materials and methods are available as supporting material on *Science* Online.
15. D. Karan *et al.*, *Evolution* **52**, 825 (1998).
16. D. Karan, P. Parkash, *Ecol. Entomol.* **23**, 391 (1998).
17. A. Addo-Bediako, S. L. Chown, K. J. Gaston, *J. Insect Physiol.* **47**, 1377 (2001).
18. L. Partridge, N. Prowse, P. Pignatelli, *Proc. R. Soc. London Ser. B* **266**, 255 (1999).
19. L. G. Harshman, A. A. Hoffmann, *Trends Ecol. Evol.* **15**, 32 (2000).
20. R. Frankham, J. D. Ballou, D. A. Briscoe, *Introduction to Conservation Genetics* (Cambridge Univ. Press, Cambridge, 2002).
21. A. A. Hoffmann, in *Adaptive Genetic Variation in the Wild*, T. A. Mousseau, B. Sinervo, J. A. Endler, Eds. (Oxford Univ. Press, New York, 2000), pp. 200–218.
22. D. A. Roff, *Evolutionary Quantitative Genetics* (Chapman & Hall, New York, 1997).
23. M. Sgrò, L. Partridge, *Am. Nat.* **156**, 341 (2000).
24. A. A. Hoffmann, R. Hallas, C. Sinclair, L. Partridge, *Evolution* **55**, 436 (2001).
25. D. H. Reed, R. Frankham, *Evolution* **55**, 1095 (2001).
26. F. Baer, J. Travis, *Evolution* **54**, 238 (2000).
27. K. A. Crandall, C. R. P. Binida-Edmonds, G. M. Mace, R. K. Wayne, *Trends Ecol. Evol.* **15**, 290 (2000).

28. We thank the Australian Research Council for financial support via its Special Research Centre Scheme, as well as the Department of Education, Training and Youth Affairs for molecular infrastructure funding, C. Sgrò and A. Weeks for comments on the manuscript, and M. Higgie for some field lines.

Supporting Online Material
www.sciencemag.org/cgi/content/full/301/5629/100/DC1
Materials and Methods

10 March 2003; accepted 6 May 2003

Inferring Genetic Networks and Identifying Compound Mode of Action via Expression Profiling

Timothy S. Gardner,[1]* Diego di Bernardo,[1,2]* David Lorenz,[1] James J. Collins[1]†

The complexity of cellular gene, protein, and metabolite networks can hinder attempts to elucidate their structure and function. To address this problem, we used systematic transcriptional perturbations to construct a first-order model of regulatory interactions in a nine-gene subnetwork of the SOS pathway in *Escherichia coli*. The model correctly identified the major regulatory genes and the transcriptional targets of mitomycin C activity in the subnetwork. This approach, which is experimentally and computationally scalable, provides a framework for elucidating the functional properties of genetic networks and identifying molecular targets of pharmacological compounds.

Efforts to systematically define the organization and function of gene, protein, and metabolite networks include experimental and computational methods for identifying molecular interactions (*1–3*), global structural properties (*4*, *5*), metabolic limits (*6*), and regulatory modules and characteristics (*7–9*). These methods have provided valuable insights in many applications, but they often provide only structural information or require extensive quantitative information, which is not generally available, particularly for larger regulatory networks. In previous computational studies (*10–12*), alternative methods have been proposed that would enable rapid deduction of network connectivity and functional properties solely from temporal gene-expression data. However, the acquisition of adequate temporal expression data remains difficult, and the practical utility of such approaches has not been determined.

Here, we present a rapid and scalable method that enables construction of a first-order predictive model of a gene and protein regulatory network using only steady-state expression measurements and no previous information on the network structure or function. We use multiple linear regression to determine the model from RNA expression changes resulting from a set of steady-state transcriptional perturbations. The model can be used to identify the regulatory role of individual genes in the network, useful control points in the network, and genes that directly mediate a pharmaceutical compound's bioactivity in the cell. The method, called network identification by multiple regression (NIR), is derived from a branch of engineering called system identification (*13*), in which a model of the connections and functional relations between elements in a network is inferred from measurements of system dynamics (e.g., the response of genes and proteins to external perturbations).

To apply a system-identification method, we assume that the behavior of a gene, protein, and metabolite regulatory network can be modeled by a system of nonlinear differential equations (*14*, *15*). Near a steady-state point (e.g., when gene expression does not change substantially over time), such a nonlinear system may be approximated to the first order by a linear system of equations describing the rate of accumulation of each network species resulting from a transcriptional perturbation:

$$d\mathbf{x}/dt = \mathbf{A}\mathbf{x} + \mathbf{u} \qquad (1)$$

where $\mathbf{x}$ is a vector representing the concentrations of N RNAs, proteins, and metabolites in the network; $d\mathbf{x}/dt$ represents the rate of accumulation of the species in $\mathbf{x}$; $\mathbf{u}$ is a vector representing an external perturbation to the rate of accumulation of the species in $\mathbf{x}$; and $\mathbf{A}$, the network model, is an $N \times N$ matrix of coefficients describing the regulatory interactions between the species in $\mathbf{x}$. Next, we identify the coefficients of $\mathbf{A}$ using only RNA expression changes that result from steady-state transcriptional perturbations. Because we measure RNA but not protein or metabolite species in this study, variables representing proteins and metabolites are not explicitly represented in the network model. Thus, regulatory connections in the model are not, in general, physical connections; rather, they represent effective functional relations between transcripts.

Under the steady-state assumption ($d\mathbf{x}/dt = 0$), Eq. 1 reduces to $\mathbf{A}\mathbf{x} = -\mathbf{u}$. To identify the network model, we could, in principle, make N distinct perturbations, $\mathbf{u}$, to the RNAs in a particular network, recover N sets of RNA concentrations, $\mathbf{x}$, and solve directly for $\mathbf{A}$ (*16*). How-

[1]Center for BioDynamics and Department of Biomedical Engineering, Boston University, 44 Cummington Street, Boston, MA 02215, USA. [2]Telethon Institute for Genetics and Medicine (TIGEM), Via P. Castellino 111, 80131, Naples, Italy.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: jcollins@bu.edu