

NEWS AND VIEWS

PERSPECTIVE

Annotating *ebony* on the fly

MICHAEL H. KOHN* and PATRICIA J. WITTKOPP†

*Department of Ecology and Evolutionary Biology, Institute of Biotechnology & Bioengineering, Rice University, MS 170, 205 A Anderson Biology Laboratory, 6100 Main Street, Houston, Texas 77005, USA, †Department of Ecology and Evolutionary Biology, Department of Molecular, Cellular, and Developmental Biology, University of Michigan, 1061 Natural Science Building, 830 North University Avenue, Ann Arbor, Michigan 48109-1048, USA

Abstract

The distinctive black phenotype of *ebony* mutants has made it one of the most widely used phenotypic markers in *Drosophila* genetics. Without doubt, *ebony* showcases the fruits of the fly community's labours to annotate gene function. As of this writing, FlyBase lists 142 references, 1277 fly stocks, 15 phenotypes and 44 alleles. In addition to its namesake pigmentation phenotype, *ebony* mutants affect other traits, including phototaxis and courtship. With phenotypic consequences of *ebony* variants readily apparent in the laboratory, does natural selection also see them in the wild? In this issue of *Molecular Ecology*, Pool & Aquadro investigate this question and found signs of natural selection on the *ebony* gene that appear to have resulted from selection for darker pigmentation at higher elevations in sub-Saharan populations of *Drosophila melanogaster*. Such findings from population genomic analysis of wild-derived strains should be included in gene annotations to provide a more holistic view of a gene's function. The evolutionary annotation of *ebony* added by Pool & Aquadro substantiates that pigmentation can be adaptive and implicates elevation as an important selective factor. This is important progress because the selective factors seem to differ between populations and species. In addition, the study raises issues to consider when extrapolating from selection at the molecular level to selection at the phenotypic level.

Pool & Aquadro (2007) scored black colouration of the fourth abdominal segment in > 350 female flies from sub-Saharan strains. Of the geographical factors they tested for correlation with dark coloration, only elevation was significant. Using the tools of fly genetics to examine the effects of individual chromosomes, they found that genetic factor(s) underlying the coloration difference between populations mapped to the X and third chromosomes. These chromosomes contain some of the best-characterized pigmentation genes: *yellow* and *tan* on the X, and *ebony* on the third chromosome. The authors chose to focus on *ebony*, sequencing this gene in strains of *Drosophila melanogaster* from Kenya and Uganda. Population genetic analysis revealed that the upstream sequences of *ebony* show signs of a gene that has been, or currently is, under selection. This region may contain *cis*-regulatory sequences that affect transcription of *ebony*. Darker cuticle pigmen-

tation, presumably caused by polymorphisms in *ebony*, may be an adaptation of the cold-blooded fly to cooler climates; dark colour helps an insect take up more of the sun's energy (Wittkopp *et al.* 2003).

Thus, we could now go ahead and add another line to the annotation file of *ebony*, perhaps sounding as follows: 'under natural selection in *D. melanogaster* living at higher elevations in Uganda, Africa'. Currently, annotation of the fly genome is done primarily at the computer, bench and in the fly room. Without the information gained through these endeavours, not much else would be possible. However, even when annotations are considered 'complete' by the bioinformaticians, molecular biologists, and geneticists, a void remains. Studies such as that by Pool & Aquadro (2007) illustrate how this void can be filled by combining phenotypic analysis with evolutionary annotations obtained by population genomics; the '-omics' offshoot of population genetics.

Surveys of genetic polymorphism across the genome, in specific genomic regions or in candidate genes for the

Correspondence: Michael H. Kohn, Fax: +1 713 348 5232; E-mail: hmkohn@rice.edu

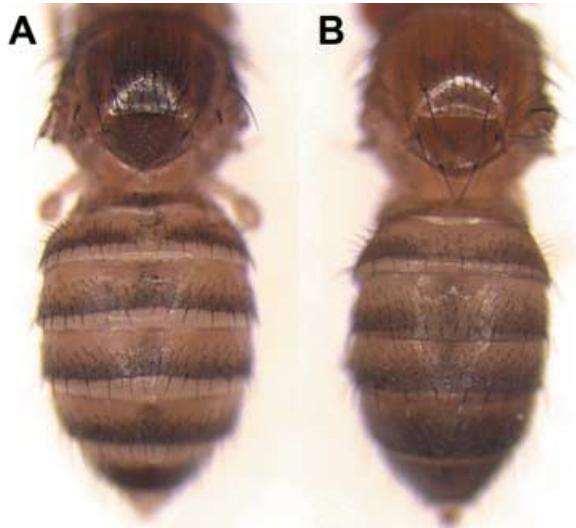


Fig. 1 Similar darkened phenotypes of protein and regulatory mutations in *ebony*. *Ebony* mutations in a TM2/TM6b balancer genetic background (A) and candidate regulatory mutations of *ebony* in a strain from Uganda (Ug1) (B). Wings were removed to show dorsal abdomen. (Picture credit: P.J. Wittkopp, fly strain Ug1 from J Pool)

telltale signs of natural selection, are one efficient way to obtain such evolutionary annotations. Typically, a loss of genetic variation, pronounced stretches of linkage disequilibrium and haplotype structure, a surplus of derived mutations segregating at high frequency, and population genetic structure can be used to infer non-neutral evolution (Storz 2005). It is then possible to take advantage of more traditional annotations of gene function to generate hypotheses about the specific phenotype under selection (Kohn *et al.* 2000). A careful consideration of a gene's function in the laboratory can minimize the risk of focusing on one of the outlier loci expected to emerge from any study under any evolutionary/demographic scenario (Thornton & Jensen 2007). Moreover, large genomic windows often emerge from such studies because of genetic hitchhiking and/or haplotype structure of the genome, making it difficult to identify the actual target(s) of selection and to document their possibly subtle functional consequences (Greenberg & Wu 2005). Functional annotations are critical for selecting candidates within a large region, but these selections must still be viewed with caution until our understanding of the genotype–phenotype relationship (especially in natural populations) is more complete.

The study by Pool & Aquadro (2007) is instructive with respect to the apparent dichotomy of mutational effects on fitness studied in the laboratory and their counterparts segregating in the wild. One of the many lessons learned from almost one century of painstaking work in the fly room is that there are many ways to make a darker fly, but that most of them adversely affect other phenotypes (Wittkopp

et al. 2003). In natural populations, negative pleiotropic side-effects may offset otherwise adaptive changes in pigmentation genes. Mutations studied in the laboratory are not expected to capture the mutational spectrum found in the wild, and it is the latter that is needed to formulate hypotheses as to how evolution might progress. Considering what is known about laboratory mutants of *ebony*, we would predict that adaptive pigmentation at higher elevations initially should come at a significant fitness cost, requiring the (delayed) evolution of modifying mutations.

However, there are also plausible mutational changes that may not have required crossing a fitness valley while climbing a mountain. Like most genes, *ebony* expression is controlled by multiple, independent, tissue-specific *cis*-regulatory elements, which enable regulatory mutations to overcome pleiotropic constraint by improving one particular detail (e.g. body colour) with minimal disruptions to other developmental duties of the gene. Indeed, experimental manipulation of *ebony* expression, alone or with other pigmentation genes such as *yellow*, is sufficient to alter melanin formation with no readily apparent fitness loss in laboratory stocks (Fig. 1). Pool & Aquadro found evidence of selection in an upstream, noncoding region of *ebony*, consistent with the idea that polymorphisms in *cis*-regulatory regions are an important source of adaptation (Caroll *et al.* 2005).

As Pool & Aquadro (2007) have rightfully cautioned, when extrapolating from the genetic signature of selection at the molecular level to natural selection on the phenotype, a few issues deserve consideration. First, selection for dark pigmentation by cooler climate cannot be a universal explanation for variable pigmentation. Cosmopolitan flies are lighter in colour even though they live in the cooler latitudes of Europe. The dark *ebony* alleles may have been lost during the founding of cosmopolitan populations or these populations may have adapted to cooler climates using different genes and/or different physiological mechanisms. Sex-specific effects of the one wildtype *ebony* allele from Uganda described in FlyBase also suggest that more complex selective scenarios should be considered.

Second, there are issues with inferences of selection on a phenotype via its most visible function inferred from laboratory stocks, here dark pigmentation. Let us examine the other, less glamorous effects *ebony* mutants can have: some *ebony* alleles show increased survival under nutrition stress. Could it be, then, that food availability might have been an important factor enabling the invasion of higher elevations and the dark colour is a pleiotropic by-product, which, on its own, is of no selective significance? Moreover, even for well-studied genes such as *ebony*, novel alleles continue to be identified in wild flies although their phenotypes remain to be studied (Weisman *et al.* 2000).

Although we lack clear expectations regarding the population genomic signatures on regulatory sequences and

genetic networks, the findings by Pool & Aquadro (2007) are consistent with partial selective sweeps or a balanced polymorphism, the latter of which has been inferred from laboratory populations containing both *ebony* laboratory mutant strains and wildtype strains (e.g. Zurcher 1963). Furthermore, determining the mode of selection operating in regulatory regions can be tedious (Ludwig 2002): *cis*-regulatory sequences in the often-vast noncoding regions of genes are diffuse, dispersed, modular, context-dependent and small (Fessele *et al.* 2002), leaving analyses with little power and without delineated functional and nonfunctional classes of sites to contrast (Eyre-Walker 2006). Finally, given the known interactions between *ebony* and *yellow*, *ebony* variants may interact epistatically with other alleles in the Ugandan population; effective selection for genes on the X-chromosome may require the presence of a certain *ebony* haplotype on the third-chromosome. Exactly such an interaction has been observed between a *yellow* transgene and a third chromosome modifier in *D. melanogaster* laboratory strain, although this modifier does not appear to be *ebony* (Wittkopp *et al.* 2002).

Evolutionary annotations are likely to send researchers back to the fly room to test specific hypotheses using both the latest techniques and classical genetic analysis. For example, allelic replacement — the gold-standard test of an allele's effects — can now be performed (Greenberg & Wu 2005). This underscores the importance of choosing systems tractable both in the laboratory and in the wild, and pigmentation seems to be an excellent choice. Perhaps the largest hole in understanding *Drosophila* biology is its ecology (cf. Powell 1997), and researchers should consider going out into the field armed with Endler's (1986) list of ways to document natural selection in the wild.

References

- Carroll SB, Grenier JK, Waatherbee SD (2005) *From DNA to Diversity*, 2nd edn, Blackwell Publishing, Malden, Massachusetts.
- Endler JA (1986) *Natural Selection in the Wild*. Princeton University Press, Princeton, New Jersey.
- Eyre-Walker A (2006) The genomic rate of adaptive evolution. *Trends in Ecology & Evolution*, **21**, 569–575.
- Fessele S, Maier H, Zischek C, Nelson PJ, Werner T (2002) Regulatory context is a crucial part of gene function. *Trends in Genetics*, **18**, 60–63.
- Greenberg AJ, Wu C-I (2005) Molecular genetics of natural populations. *Molecular Biology and Evolution*, **23**, 883–886.
- Kohn MH, Pelz H-P, Wayne RK (2000) Natural selection mapping of the warfarin resistance gene. *Proceedings of the National Academy of Sciences, USA*, **97**, 7911–7915.
- Ludwig MZ (2002) Functional evolution of noncoding DNA. *Current Opinions in Genetics and Development*, **12**, 634–639.
- Pool JE, Aquadro CF (2007) The genetic basis of adaptive pigmentation variation in *Drosophila melanogaster*. *Molecular Ecology*, doi: 10.1111/j.1365-294X.2007.03304.x
- Powell JR (1997) *Progress and Prospects in Evolutionary Biology: The Drosophila Model*. Oxford University Press, New York.
- Storz JF (2005) Using genome scans of DNA polymorphism to infer adaptive population divergence. *Molecular Ecology*, **14**, 671–688.
- Thornton KR, Jensen JD (2007) Controlling the false-positive discovery rate in multilocus genome scans. *Genetics*, **175**, 737–750.
- Weisman NY, Erokhina ID, Zakharov IK (2000) Spectrum of visible mutations isolated out of wild populations of *Drosophila melanogaster* in 2000. *Drosophila Information Service*, **84**, 82–84.
- Wittkopp PJ, Carroll SB, Kopp A (2003) Evolution in black and white: genetic control of pigment patterns in *Drosophila*. *Trends in Genetics*, **9**, 495–504.
- Wittkopp PJ, Vaccaro K, Carroll SB (2002) Evolution of yellow gene regulation and pigmentation in *Drosophila*. *Current Biology*, **12**, 1547–1556.
- Zurcher C (1963) Der Faktor *e* by *Drosophila melanogaster*: Lokalisation, Frequenzänderungen in Massenzuchten und Lebensleitungen seiner Träger in Vergleich mit einem Wildstamm. *Genetica*, **34**, 1–33.

Patricia J. Wittkopp studies genetic changes responsible for phenotypic differences within and between species. Molecular mechanisms underlying the evolution of gene expression and *Drosophila* pigmentation are currently under investigation. Michael H. Kohn is studying evolutionary genomics, conservation genetics, and medical genetics. The detection of adaptations at the level of genes is one main area of interest that is studied in rat populations resistant to anticoagulant rodenticides (warfarin).
