

Genealogical portraits of speciation in montane grasshoppers (genus *Melanoplus*) from the sky islands of the Rocky Mountains

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Grasshoppers in the genus *Melanoplus* have undergone a radiation in the 'sky islands' of western North America, with many species originating during the Pleistocene. Despite their recent origins, phylogenetic analyses indicate that all the species exhibit monophyletic or paraphyletic gene trees. The objectives of this study were to determine whether the monophyletic genealogies are the result of a bottleneck at speciation and to investigate the extent to which the different phylogenetic states of eight species (i.e. monophyletic versus paraphyletic gene trees) can be ascribed to the effects of speciation. A coalescent simulation was used to test for a bottleneck at speciation in each species. The effective population sizes and demographic histories of species were compared across taxa to evaluate the possibility that the paraphyly versus monophyly of the species reflects differential rates of lineage loss rather than speciation mode. While coalescent analyses indicate that the monophyly of *Melanoplus* species might not be indicative of bottlenecks at speciation, the results suggest that the paraphyletic gene trees may reflect the demography of speciation, involving localized divergences in the ancestral species. With respect to different models of Pleistocene divergence, the data do not support a model of founder-effect speciation but are compatible with divergence in allopatric refugia.

Keywords: bottlenecks; coalescence; gene genealogy; historical demography; Pleistocene; speciation

1. INTRODUCTION

Gene genealogies are often used to study the geography of species divergence (Avice 1989; Harrison 1991; Crandall & Templeton 1993; Riddle 1996) but they can also provide information about the demography of speciation. For example, using models of evolutionary processes based on coalescent theory (Kingman 1982), gene trees can be used to test different hypotheses about the speciation process, such as whether there was a bottleneck at speciation (e.g. Takahata 1993; Eyre-Walker *et al.* 1998; Klein *et al.* 1998; Knowles *et al.* 1999).

A gene genealogy undergoes a progression from an initial state of polyphyly to paraphyly and eventually monophyly as the time since speciation increases (Neigel & Avice 1986; Ball *et al.* 1990; Avice 1994). This transition to monophyly requires $4N_c$ generations, on average, where N_c is the effective population size (Hudson 1990; Chesser & Baker 1996; for exceptions, see Hoelzer 1997). In addition to the influence of the species' historical population size, the mode of speciation also affects a gene genealogy. A bottleneck at speciation can produce a monophyletic gene tree (Neigel & Avice 1986), whereas a paraphyletic gene tree occurs if a descendant species arises from one of several populations of the ancestral species (i.e. from a subset of the ancestral species' gene tree), such as during peripatric speciation (e.g. Avice *et al.* 1983; Hey & Kliman 1993). However, although monophyletic and paraphyletic gene trees may be consistent with particular modes of speciation, they do not necessarily reflect the demography of speciation (Knowles *et al.*

1999). A monophyletic gene tree may arise if there has been sufficient time for coalescence of lineages due to a species' small effective population size. Similarly, a paraphyletic gene tree may reflect the retention of ancestral polymorphism because of a species' large effective population size (Maddison 1997). Therefore, since the speciation process and the demographic history of species affect the rate of transition of gene genealogies, both influences should be considered.

This study addresses the extent to which the mode of speciation in *Melanoplus* grasshoppers has left a signature on the species' genealogies, and whether speciation in this group involved bottlenecks. It has previously been shown that the *Melanoplus* species inhabiting the 'sky islands' of the northern Rocky Mountains originated during the Pleistocene (Knowles 1999, 2000a; Knowles & Otte 1999). Despite their recent origins, many of the species exhibit monophyletic gene trees (table 1; this study) suggesting that they may have experienced a bottleneck at speciation. Moreover, bottlenecks are a common feature of speciation models involving displacement of species into glacial refugia (e.g. Mengel 1964; Hewitt 1996, 2000). The northern Rocky Mountain region was dominated by the Cordilleran ice sheet and a complex of mountain and valley glaciers during the Pleistocene (Hollin & Schilling 1981; Mayweski *et al.* 1981), which displaced species to ice-free refugia (Pielou 1991; Elias 1996). However, not all the *Melanoplus* species under study have monophyletic gene trees (table 1). Thus, the goal of this study was to determine whether the mode of speciation differs between species. Coalescent simulations were used to evaluate the likelihoods that the monophyletic genealogies reflect bottlenecks at speciation. Estimates of the effective population sizes and consideration of the historical demographies of the species were used to

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Table 1. *Phylogenetic status (from Knowles 2000a) and estimates of genetic diversities (i.e. π and $\hat{\theta}$), effective population sizes (N_f), Tajima's D and time of origin of each species (estimates were made using the program SITES (Wakeley & Hey 1997) and MEGA (Kumar et al. 1993))*

(The number of specimens sequenced is given in parentheses. Significant values of Tajima's D (i.e. values outside the 95% confidence interval of the neutral expectation based on table 1 in Tajima (1989)) are shown in bold. The range of divergence times encompasses the standard errors calculated for the average pairwise differences between species assuming one generation per year and using Kimura's two-parameter model to correct for multiple substitutions (Kimura 1980).)

species	π bp ^a	$\hat{\theta}$ bp ^b	Tajima's D	N_f ^c	divergence time (Myr)	phylogenetic status
<i>M. oregonensis</i> (124)	0.1957	0.2977	− 1.9159	850 000–1 200 000	1.10–1.64	monophyletic
<i>M. marshalli</i> (5)	0.1926	0.2426	− 1.4190	837 400–1 100 000	1.10–1.64	monophyletic
<i>M. indigenus</i> (3)	0.1442	0.1534	−0.7850	627 000–667 000	0.69–1.08	monophyletic
<i>M. crux</i> (9)	0.1025	0.1377	−1.2903	512 500–688 500	0.69–1.08	paraphyletic
<i>M. missouli</i> (6)	0.0902	0.1288	− 1.9067	392 200–560 000	0.50–0.82	monophyletic
<i>M. triangularis</i> (8)	0.0918	0.0962	−0.4268	399 100–418 300	0.50–0.82	paraphyletic
<i>M. montanus</i> (4)	0.0834	0.0894	−0.6885	362 600–388 700	0.91–1.40	monophyletic
<i>M. moyense</i> (4)	0.0589	0.0658	− 1.6758	256 000–286 000	0.91–1.40	monophyletic

^a π , the average number of pairwise differences between sequences (Tajima 1983).

^b $\hat{\theta}$, based on the number of segregating sites (Watterson 1975).

^c N_f , derived from the expected value of $2N_f\mu$ for both π and $\hat{\theta}$.

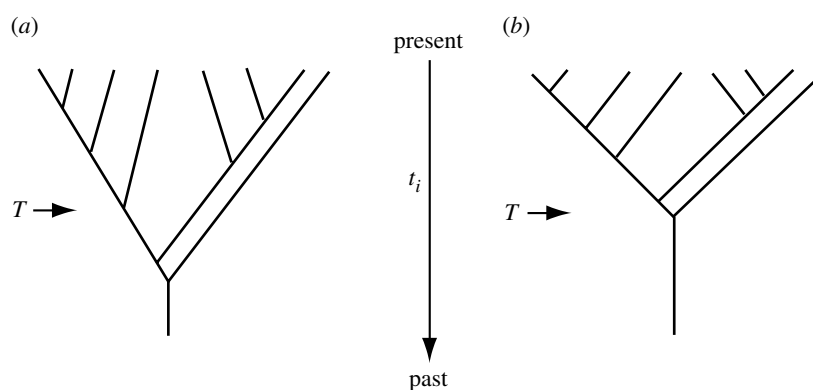


Figure 1. The two models used in the coalescent simulation. Under the bottleneck model (b) at time $t > T$, the i lineages ancestral to the n sampled sequences coalesce to a single ancestral lineage with a probability of 1, whereas under the constant-population-size model (a) the lineages continue to coalesce at the rate of $i(i-1)/2N_e$ (Hudson 1990). Consequently, in the bottleneck model (b) it is assumed that a single individual gave rise to the species, followed by an immediate expansion such that the effective population size, N_e , immediately following the speciation event is the same for both models. However, moderately larger numbers of founders or lower rates of growth will give similar results (B. Rannala, unpublished data).

evaluate whether different rates of lineage loss, rather than the mode of speciation, account for the phylogenetic status of the genealogies.

2. MATERIAL AND METHODS

(a) *The study system*

The study focused on eight grasshopper species in the genus *Melanoplus* (Stal) (Orthoptera: Acrididae: Melanoplinae: Indigenus and Montanus species groups) distributed in the northern Rocky Mountains (Scudder 1898; Knowles 1999; Otte 2001). The number of specimens sequenced, estimates of divergence times and phylogenetic status for each species are given in table 1. A 1300 base-pair fragment of cytochrome oxidase I mitochondrial DNA was sequenced for each individual (Knowles 2000a,b). The phylogenetic analyses are described by Knowles (2000a,b). The phylogenetic status of each species was consistent across all analyses (Knowles 2000a,b); data were

analysed using parsimony, neighbour-joining and maximum-likelihood methods (PAUP*, Swofford 1998). A rate of divergence of 2.3% per million years (Myr) (Brower 1994) was used to calculate species' divergence times, T , and to estimate the mutation rate per base pair per sequence per generation, μ . This rate of molecular evolution is similar to other estimates (e.g. Brown *et al.* 1979).

(b) *Demographic and coalescent analyses*

To determine whether different rates of lineage loss account for the monophyly versus paraphyly of the species, the effective population size and demographic history of each species were compared. The effective population size of each species was derived from estimates of their respective genetic diversities (i.e. from π and $\hat{\theta}$; table 1). The frequency distribution of pairwise differences between sequences (i.e. the mismatch distribution) was calculated for each species, and then the shapes of the distributions were compared to detect variation in the species'

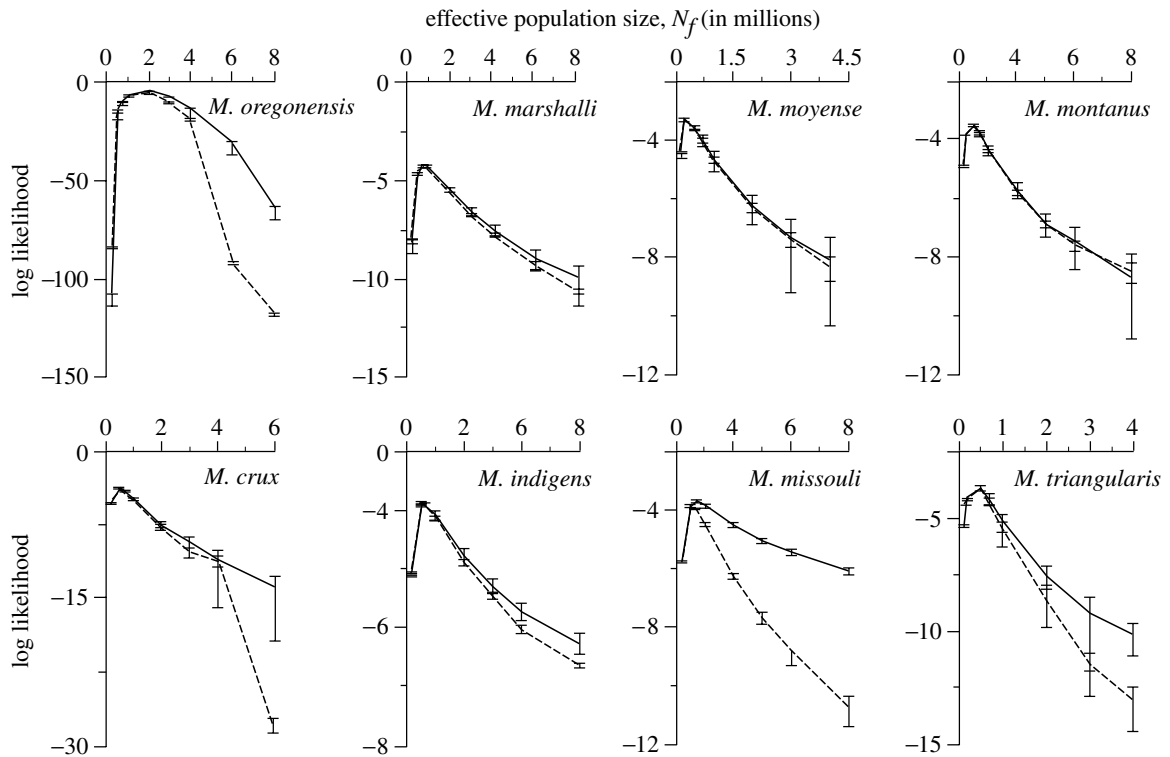


Figure 2. Log-likelihood surface (with standard errors) of each of the species' gene genealogies under the two coalescent models: a constant population size with no speciation bottleneck (solid line) and a dramatic bottleneck at speciation (dashed line). For each set of parameter values 10 000 replicates were simulated.

demographic histories (Slatkin & Hudson 1991; Marjoram & Donnelly 1994). The fit of each species to Wakeley & Hey's (1997) model of population expansion based on the number of segregating sites was also examined. Tajima's D (Tajima 1989) was also calculated for each species and compared across species to provide an additional comparison of their demographic histories. While this measure of variation can be used to examine the history of selection, it can also be used to make inferences about population demography. Tajima's D is expected to be negative under a model of population expansion and positive under population subdivision (e.g. Aris-Brosou & Excoffier 1996).

A likelihood approach was used to evaluate the genetic evidence for a bottleneck associated with speciation in each species. A coalescent process (Kingman 1982) was used to model the species' genealogies expected under two different demographic scenarios: first, a constant population size with no speciation bottleneck (figure 1a) and, second, a dramatic bottleneck at speciation (figure 1b). The probability of observing S segregating sites in a sample of n sequences (the likelihood, when treating S as the observed data) was calculated under the two models for a range of population sizes, assuming an infinite-sites model of sequence mutation. The probability of S is

$$\Pr(S|\mu, T, N) = \int_{\mathbf{t}} \Pr(S|\mu, \mathbf{t}) \Pr(\mathbf{t}|N, T) d\mathbf{t}, \quad (1)$$

where μ is the per-sequence mutation rate (i.e. the per-site mutation rate multiplied by the number of sites in the sequence) and $\mathbf{t} = \{t_n, t_{n-1}, \dots, t_2\}$, is a vector of the coalescence times, where t_i is the waiting time for n sequences to coalesce to $i-1$ ancestral sequences. Monte Carlo integration and simulation

from the coalescent process $\Pr(\mathbf{t}|N, T)$ was used to evaluate the above integral, where T is the time of species divergence (program provided by B. Rannala).

3. RESULTS

Estimates of π and $\hat{\theta}$, as well as Tajima's D , for each species are presented in table 1. The paraphyletic species (i.e. *M. crux* and *M. triangularis*) clearly do not have larger effective population sizes than most of the monophyletic species (table 1). This conclusion is independent of the different estimates of N_e ; in general, estimates of N_e derived from π and $\hat{\theta}$ (table 1) correspond to the maximum-likelihood estimates from the coalescent simulations (figure 2), with the exception of *M. oregonensis*.

Demographic histories do not vary in an obvious way across most species. While Tajima's D is not significant in all species, it is consistently negative (table 1). Moreover, the frequency spectrum of pairwise differences for each species was multimodal, with the exception of a unimodal distribution in *M. oregonensis* (figure 3). However, a multimodal distribution can result from a variety of demographic scenarios (Slatkin & Hudson 1991; Marjoram & Donnelly 1994), and the small sample sizes (see table 1) in some of the species may have affected the distribution.

M. oregonensis fits a population-expansion model based on the number of segregating sites (Wakeley & Hey 1997), and the unimodal distribution of pairwise differences and significantly negative Tajima's D are consistent with a model of population expansion in *M. oregonensis* (Slatkin & Hudson 1991; Rogers & Harpending 1992; Aris-Brosou & Excoffier 1996). Given that the species studied here are

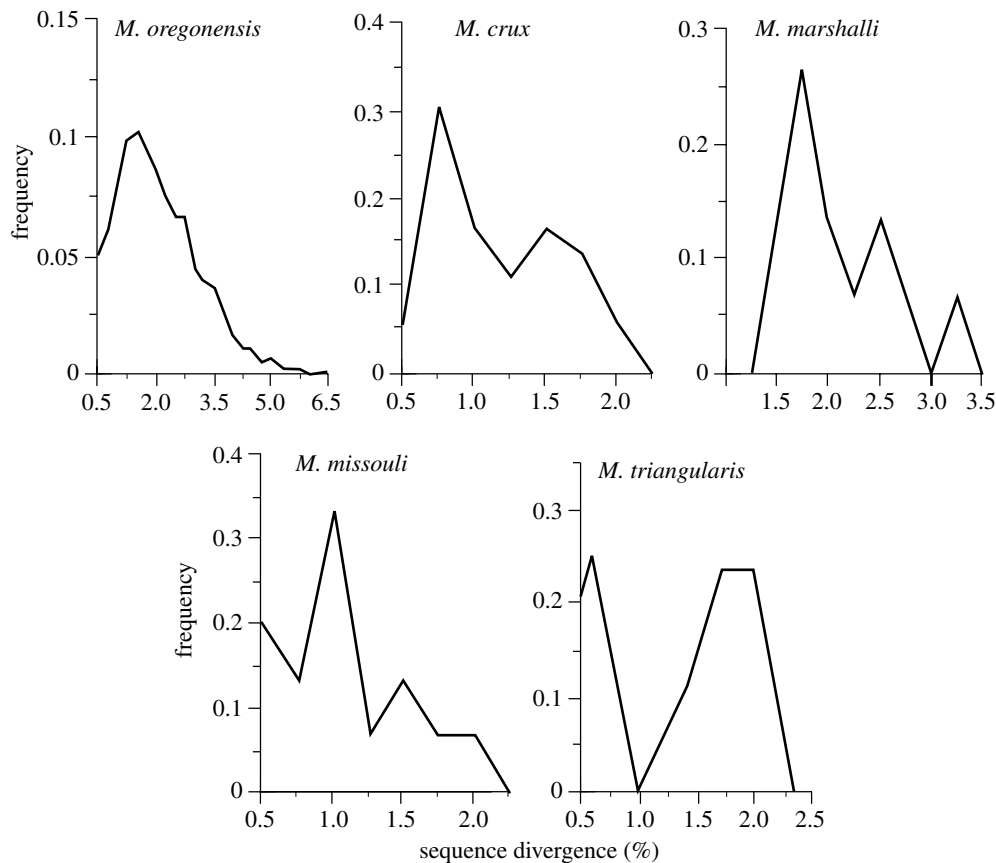


Figure 3. Observed frequency distributions of pairwise differences between sequences for each species. *M. montanus*, *M. moyense* and *M. indigens* are not included because of the small number of sequences in each species. To correct for multiple substitutions, Kimura's two-parameter model (Kimura 1980) was used to calculate pairwise genetic distances.

distributed in previously glaciated areas (Knowles 1999), this result is not unexpected. However, it does not indicate that the species is panmictic (Knowles 2000b). Depending on the level of gene flow, even a population with substructure can exhibit a unimodal distribution (Hudson 1990; Bertorelle & Slatkin 1995).

The coalescent simulations indicate that the likelihood of a bottleneck at speciation is not higher than the likelihood of no bottleneck (i.e. the confidence intervals of the highest log-likelihood values overlap between the two models in all species; figure 2). Furthermore, the lack of a significant difference between the two models shows that there has been sufficient time for lineage sorting to occur, i.e. for the species' gene trees to become monophyletic. While it may be somewhat surprising that there has been sufficient time for coalescence of lineages in species that originated around 1 Myr ago (e.g. *M. oregonensis* and *M. marshalli*), as well as in species that originated as recently as 500 000 years ago (e.g. *M. indigens* and *M. missouli*), the more recently derived species do have correspondingly smaller effective population sizes compared to the older species (table 1).

4. DISCUSSION

These analyses indicate that the origin of the *Melanoplus* species studied here did not necessarily involve bottlenecks. The coalescent simulations show that the monophyletic gene trees are consistent with the hypothesis that

there has been sufficient time for coalescence of lineages within species (figure 2). Moreover, since there are no obvious differences in the effective population sizes or demographic histories between the species with paraphyletic and monophyletic gene trees (table 1 and figure 3), the paraphyly is not attributable to a slower rate of progression towards monophyly. Consequently, it is more likely that the paraphyletic genealogies reflect a mode of speciation in which descendant species arose from one of several populations of the ancestral species (e.g. Harrison 1991).

Thus, as a complement to studies that have demonstrated that high genetic diversities in recently derived species do not preclude the possibility of a bottleneck at speciation (e.g. Eyre-Walker *et al.* 1998), the present findings argue that monophyletic genealogies, even in recently derived species, do not necessarily imply a bottleneck at speciation (see also Knowles *et al.* 1999). Furthermore, these results, in conjunction with phylogeographic analyses of population divergence in *Melanoplus* (Knowles 2000b), provide not only a clearer portrait of speciation in these grasshoppers but also offer insight into the general process of Pleistocene speciation.

The picture that emerges from both the population- and species-level approaches is one of divergence in allopatric refugia (e.g. Mengel 1964; Hewitt 1996) rather than speciation by bottlenecks, such as postulated by founder-effect models (e.g. Mayr 1954; Templeton 1980; Carson 1982; Gavrilets & Hastings 1996). However, because of the frequency of glacial cycles (Bartlein &

Prentice 1989; Dansgaard *et al.* 1993), and consequently the short time interval separating shifts in species distributions (Roy *et al.* 1996), Pleistocene speciation must involve the rapid evolution of reproductive isolation (Knowles 1999, 2000a; Dynesius & Jansson 2000). Indeed, while regional structuring of population variation in *Melanoplus* supports the hypothesis that divergence occurred in allopatric refugia, evidence of sporadic gene flow indicates that these differences will be lost during interglacial expansion without a mechanism to prevent population mixing (Knowles 2000b).

In the absence of evidence to support drift-induced rapid divergence, some other process, such as selection, and specifically sexual selection, may account for the rapidity of the speciation process in *Melanoplus* species originating during the Pleistocene. The *Melanoplus* species studied here occupy similar habitats and are morphologically very similar except in the shape of the male genitalia (Knowles 1999; Otte 2001). Male genitalia in insects are posited to be under sexual selection and characters under sexual selection can evolve rapidly (Eberhard 1985, 1993, 1996; Arnqvist 1998). Differences in male genitalia can also play an important role in reproductive isolation (Eberhard 1996; Arnqvist & Danielsson 1999).

In addition to examples of species divergence in association with long-standing geographic barriers (e.g. Avise 1994), the role of transient allopatry coupled with natural or sexual selection in speciation has recently been recognized in a variety of species (e.g. McCune 1997; Hellberg 1998; Orr & Smith 1998; Schluter 1998). Thus, it is highly likely that speciation in *Melanoplus* is a product of sexual selection and divergence initiated in allopatric glacial refugia.

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REFERENCES

- Aris-Brosou, S. & Excoffier, L. 1996 The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism. *Mol. Biol. Evol.* **13**, 494–504.
- Arnqvist, G. 1998 Comparative evidence for the evolution of genitalia by sexual selection. *Nature* **393**, 784–786.
- Arnqvist, G. & Danielsson, I. 1999 Copulatory behavior, genital morphology, and male fertilization success in water striders. *Evolution* **53**, 147–156.
- Avise, J. C. 1989 Gene trees and organismal histories: a phylogenetic approach to population biology. *Evolution* **43**, 1192–1208.
- Avise, J. C. 1994 *Molecular markers, natural history, and evolution*. London: Chapman & Hall.
- Avise, J. C., Shapira, J. F., Daniel, S. W., Aquadro, C. F. & Lansman, R. A. 1983 Mitochondrial DNA differentiation during the speciation process in *Peromyscus*. *Mol. Biol. Evol.* **1**, 38–56.
- Ball Jr, R. M., Neigel, J. E. & Avise, J. C. 1990 Gene genealogies within the organismal pedigrees of random mating populations. *Evolution* **44**, 360–370.
- Bartlein, P. J. & Prentice, I. C. 1989 Orbital variations, climate and paleoecology. *Trends Ecol. Evol.* **4**, 195–199.
- Bertorelle, G. & Slatkin, M. 1995 The number of segregating sites in expanding human populations, with implications for estimates of demographic parameters. *Mol. Biol. Evol.* **12**, 887–892.
- Brower, A. V. Z. 1994 Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proc. Natl Acad. Sci. USA* **91**, 6491–6495.
- Brown, W. M., George Jr, M. & Wilson, A. C. 1979 Rapid evolution of animal mitochondrial DNA. *Proc. Natl Acad. Sci. USA* **76**, 1967–1971.
- Carson, H. L. 1982 Speciation as a major reorganization of polygenic balance. In *Mechanisms of speciation* (ed. C. Barigozzi), pp. 411–433. New York: Liss.
- Chesser, R. K. & Baker, R. J. 1996 Effective sizes and dynamics of uniparentally and diparentally inherited genes. *Genetics* **144**, 1225–1235.
- Crandall, K. A. & Templeton, A. R. 1993 Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* **134**, 959–969.
- Dansgaard, W. (and 10 others) 1993 Evidence of general instability of past climate from a 250-kyr ice-core record. *Nature* **364**, 218–220.
- Dynesius, M. & Jansson, R. 2000 Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch climate oscillations. *Proc. Natl Acad. Sci. USA* **97**, 9115–9120.
- Eberhard, W. G. 1985 *Sexual selection and animal genitalia*. Cambridge, MA: Harvard University Press.
- Eberhard, W. G. 1993 Evaluating models of sexual selection: genitalia as a test case. *Am. Nat.* **142**, 564–571.
- Eberhard, W. G. 1996 *Female control: sexual selection by cryptic female choice*. Princeton University Press.
- Elias, S. A. 1996 *The ice-age history of national parks in the Rocky Mountains*. Washington, DC: Smithsonian Institution Press.
- Eyre-Walker, A., Gaut, R. L., Hilton, H., Feldman, D. & Gaut, B. S. 1998 Investigation of the bottleneck leading to the domestication of maize. *Proc. Natl Acad. Sci. USA* **95**, 4441–4446.
- Gavrilets, S. & Hastings, A. 1996 Founder-effect speciation: a theoretical reassessment. *Am. Nat.* **147**, 466–491.
- Harrison, R. 1991 Molecular changes at speciation. *A. Rev. Ecol. Syst.* **22**, 281–308.
- Hellberg, M. E. 1998 Sympatric sea shells along the sea's shore: the geography of speciation in the marine gastropod *Tegula*. *Evolution* **52**, 1311–1324.
- Hewitt, G. M. 1996 Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linn. Soc.* **58**, 247–276.
- Hewitt, G. M. 2000 The genetic legacy of the Quaternary ice ages. *Nature* **405**, 907–913.
- Hey, J. & Kliman, R. M. 1993 Population genetics and phylogenetics of DNA sequence variation at multiple loci within the *Drosophila melanogaster* species complex. *Mol. Biol. Evol.* **10**, 804–822.
- Hoelzer, G. A. 1997 Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees revisited. *Evolution* **51**, 622–626.
- Hollin, J. T. & Schilling, D. H. 1981 Late Wisconsin–Weichselian mountain glaciers and small ice caps. In *The last great ice sheets* (ed. G. H. Denton & T. J. Hughes), pp. 179–198. New York: Wiley.
- Hudson, R. R. 1990 Gene genealogies and the coalescent process. *Oxf. Surv. Evol. Biol.* **7**, 1–44.
- Kimura, M. 1980 A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**, 111–120.

- Kingman, J. F. C. 1982 The coalescent. *Stochastic Process. Appl.* **13**, 235–248.
- Klein, J., Sato, A., Nagl, S. & O'hUigin, C. 1998 Molecular trans-species polymorphism. *A. Rev. Ecol. Syst.* **29**, 1–21.
- Knowles, L. L. 1999 Genealogical portraits of Pleistocene speciation and diversity patterns in montane grasshoppers. PhD dissertation, State University of New York at Stony Brook, New York.
- Knowles, L. L. 2000a Tests of Pleistocene speciation in montane grasshoppers from the sky islands of western North America (genus *Melanoplus*). *Evolution* **54**, 1337–1348.
- Knowles, L. L. 2000b Patterns of population differentiation and their implications for speciation in montane grasshoppers (genus *Melanoplus*). *Mol. Ecol.* (In the press.)
- Knowles, L. L. & Otte, D. 1999 Phylogenetic analysis of montane grasshoppers from western North America (genus *Melanoplus*, Acrididae: Melanoplineae). *Annls Entomol. Soc. Am.* **93**, 421–431.
- Knowles, L. L., Futuyma, D. J., Eanes, W. F. & Rannala, B. 1999 Insight into speciation from historical demography in the phytophagous beetle genus *Ophraella*. *Evolution* **53**, 1846–1856.
- Kumar, S., Tamura, K. & Nei, M. 1993 *MEGA: molecular evolutionary genetic analysis*. Pennsylvania State University.
- McCune, A. R. 1997 How fast is speciation? Molecular, geological, and phylogenetic evidence from adaptive radiations of fishes. In *Molecular evolution and adaptive radiation* (ed. T. J. Givnish & K. J. Sytsma), pp. 585–610. London: Cambridge University Press.
- Maddison, W. 1997 Gene trees in species trees. *Syst. Biol.* **46**, 523–536.
- Marjoram, P. & Donnelly, P. 1994 Pairwise comparisons of mitochondrial DNA sequences in subdivided populations and implications for early human evolution. *Genetics* **136**, 673–683.
- Mayr, E. 1954 Change of genetic environment and evolution. In *Evolution as a process* (ed. J. Huxley, A. C. Hardy & E. B. Ford), pp. 157–180. London: Allen and Unwin.
- Mayweski, P. A., Denton, G. H. & Hughes, T. J. 1981 Late Wisconsin ice sheets in North America. In *The last great ice sheets* (ed. G. H. Denton & T. J. Hughes), pp. 67–89. New York: Wiley.
- Mengel, R. M. 1964 The probable history of species formation in some northern wood warblers. *Living Birds* **3**, 9–43.
- Neigel, J. E. & Avise, J. C. 1986 Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. In *Evolutionary processes and theory* (ed. E. Nevo & S. Karlin), pp. 515–534. New York: Academic Press, Inc.
- Orr, M. R. & Smith, T. B. 1998 Ecology and speciation. *Trends Ecol. Evol.* **13**, 502–506.
- Otte, D. 2001 *The North American grasshoppers*, vol. 3. Cambridge, MA: Harvard University Press. (In preparation.)
- Pielou, E. C. 1991 *After the ice age: the return of life to glaciated North America*. University of Chicago Press.
- Riddle, B. R. 1996 The molecular phylogeographic bridge between deep and shallow history in continental biotas. *Trends Ecol. Evol.* **11**, 187–228.
- Rogers, A. R. & Harpending, H. 1992 Populations growth makes waves in the distribution of pairwise differences. *Mol. Biol. Evol.* **9**, 552–569.
- Roy, K., Valentine, J. W., Jablonski, D. & Kidwell, S. M. 1996 Scales of climatic variability and time averaging in Pleistocene biotas: implications for ecology and evolution. *Trends Ecol. Evol.* **11**, 458–463.
- Schluter, D. 1998 Ecological causes of speciation. In *Endless forms: species and speciation* (ed. D. J. Howard & S. H. Berlocher), pp. 114–129. New York: Oxford University Press.
- Scudder, S. H. 1898 Revision of the orthopteran group Melanopli (Acrididae) with special reference to North American forms. *Proc. US Natl Mus.* **20**, 1–421.
- Slatkin, M. & Hudson, R. R. 1991 Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics* **129**, 555–562.
- Swofford, D. L. 1998 *PAUP*: phylogenetic analysis using parsimony and other methods, beta version 4.0b1*. Sunderland, MA: Sinauer Associates.
- Tajima, F. 1983 Evolutionary relationship of DNA sequences in finite populations. *Genetics* **105**, 437–460.
- Tajima, F. 1989 Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**, 585–595.
- Takahata, N. 1993 Allelic genealogy and human evolution. *Mol. Biol. Evol.* **10**, 2–22.
- Templeton, A. R. 1980 The theory of speciation via the founder principle. *Genetics* **94**, 1011–1038.
- Wakeley, J. & Hey, J. 1997 Estimating ancestral population parameters. *Genetics* **145**, 847–855.
- Watterson, G. A. 1975 On the number of segregating sites in genetical models without recombination. *Theor. Popul. Biol.* **7**, 188–193.