were founded from a strain unable to grow on arabinose (Ara^-), and the other six were founded with a spontaneous Ara^+ mutant; the two ancestors were otherwise isogenic, and the Ara marker itself is neutral in the glucose medium[9].

Fitness assays

The protocol for estimating the competitive fitness of evolved lines relative to their ancestor has been described[9]. In brief, samples of the evolved lines (containing whatever genetic diversity was present when they were sampled) and ancestral strains were removed from the freezer and separately acclimated to the medium and culture conditions used in the evolution experiment. Each evolved line was mixed with an equal volume of the reciprocally marked ancestor, and the two types then grew and competed under the same conditions that prevailed during the evolution experiment. Initial and final densities of the two competitors were enumerated by plating cells on a tetrazolium-arabinose indicator agar that allowed them to be distinguished by the Ara marker. (A different plating procedure was used for competitions with one evolved line that no longer produced distinct colonies on the indicator agar.) The net growth rate of each competitor was calculated from the data, and the relative fitness of an evolved line is then expressed simply as the ratio of its growth rate to that of the ancestor. Assays were run in blocks with fivefold replication for all 12 lines.

Biolog assays

Catabolic diet breadth was assayed using Biolog (Hayward, California) ES plates for the two ancestral variants and three clones randomly chosen from each evolved line at generations 2,000, 10,000 and 20,000. Assays were run in three sets, each set comprising all of the clones for four lines plus three replicates of each ancestral variant. The bacteria were grown for two days in LB broth; on the next day, each culture was diluted 1:100 into fresh LB and incubated for 6 h. (LB was used instead of minimal glucose medium to avoid catabolite repression, which depresses other functions and may yield fewer positive readings[23].) The cultures were centrifuged at 12,000 *g* for 10 min and resuspended in saline to remove residual medium; this suspension was used to inoculate each well at a constant density. At 0, 4, 12, 24 and 48 h, optical densities were measured at 590 nm using an automated plate reader, and all measurements were adjusted by subtracting out the reading from the blank well. A trapezoidal area approximation[24] was used to integrate the five measurements for each well into one value, which reflects the area beneath the curve of optical density versus time; this area value is sensitive to both the rate and final level of catabolic function. Of the 95 substrates, glucose and arabinose were excluded *a priori* because glucose was the target of adaptation and arabinose use was a marker in the evolution experiment. Another 29 substrates were excluded because repeated measurements on the ancestor were statistically unreliable (coefficient of variation > 1), leaving 64 informative substrates. To test the evolutionary change in each individual catabolic function, the values for the three clones from a line at a given generation were averaged. The 12 evolved lines as a group were compared with the two ancestral variants using a two-tailed *t*-test with unequal variances (given divergence among the replicate lines) and a very stringent *P*-value of 0.0005 (to adjust for multiple tests[14]). Also, for each informative substrate, the catabolic function of an evolved clone was standardized to the ancestral value to give equal weight to all substrates, and then log-transformed to give equal weight to proportionally equivalent gains and losses of function. The anti-log of the average of these transformed values provides a measure of total catabolic function; the ancestral total equals 1.0 (by definition), whereas values less than 1.0 indicate an overall loss of function.

Received 28 March 2000; accepted 21 June 2000.

1. Mills, D. R., Peterson, R. L. & Spiegelman, S. An extracellular Darwinian experiment with a self-duplicating nucleic acid molecule. *Proc. Natl Acad. Sci. USA* **58,** 217–224 (1967).
2. Futuyma, D. J. & Moreno, G. The evolution of ecological specialization. *Annu. Rev. Ecol. Syst.* **19,** 207–233 (1988).
3. Fry, J. D. Tradeoffs in fitness on different hosts: evidence from a selection experiment with a phytophagous mite. *Am. Nat.* **136,** 569–580 (1990).
4. Bennett, A. F. & Lenski, R. E. Evolutionary adaptation to temperature. II. Thermal niches of experimental lines of *Escherichia coli*. *Evolution* **47,** 1–12 (1993).
5. Rose, M. R. & Charlesworth, B. A test of evolutionary theories of senescence. *Nature* **287,** 141–142 (1980).
6. Rose, M. R. *Evolutionary Biology of Aging* (Oxford Univ. Press, Oxford, 1991).
7. Holt, R. D. Demographic constraints in evolution: towards unifying the evolutionary theories of senescence and niche conservatism. *Evol. Ecol.* **10,** 1–11 (1996).
8. Sgrò, C. M. & Partridge, L. A delayed wave of death from reproduction in *Drosophila*. *Science* **286,** 2521–2524 (1999).
9. Lenski, R. E., Rose, M. R., Simpson, S. C. & Tadler, S. C. Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. *Am. Nat.* **138,** 1315–1341 (1991).
10. Lenski, R. E. & Travisano, M. Dynamics of adaptation and diversification: a 10,000-generation experiment with bacterial populations. *Proc. Natl Acad. Sci. USA* **91,** 6808–6814 (1994).
11. Sniegowski, P. D, Gerrish, P. J. & Lenski, R. E. Evolution of high mutation rates in experimental populations of *Escherichia coli*. *Nature* **387,** 703–705 (1997).
12. Cooper, V. S. Consequences of ecological specialization in experimental long-term evolving populations of *Escherichia coli*. Thesis, Michigan State Univ. (2000).
13. Kimura, M. *The Neutral Theory of Molecular Evolution* (Cambridge Univ. Press, Cambridge, 1983).
14. Miller, R. G. *Simultaneous Statistical Inference* (McGraw Hill, New York, 1981).
15. Cooper, V. S., Schneider, D., Blot, M. & Lenski, R. E. Mechanisms causing rapid and parallel losses of ribose catabolism in evolving populations of *E. coli* B. *J. Bacteriol.* (submitted).
16. Funchain, P. *et al.* The consequences of growth of a mutator strain of *Escherichia coli* as measured by loss of function among multiple gene targets and loss of fitness. *Genetics* **154,** 959–970 (2000).
17. De Visser, J. A. G. M., Zeyl, C. W., Gerrish, P. J., Blanchard, J. L. & Lenski, R. E. Diminishing returns from mutation supply rate in asexual populations. *Science* **283,** 404–406 (1999).
18. Szathmáry, E. Do deleterious mutations act synergistically? Metabolic control theory provides a partial answer. *Genetics* **133,** 127–132 (1993).
19. Muller, H. J. The relation of recombination to mutational advantage. *Mutat. Res.* **1,** 2–9 (1964).
20. Kondrashov, A. S. Deleterious mutations and the evolution of sexual reproduction. *Nature* **336,** 435–440 (1988).
21. Houle, D., Hoffmaster, D. K., Assimacopoulos, S. & Charlesworth, B. The genomic mutation rate for fitness in *Drosophila*. *Nature* **359,** 58–60 (1992).
22. Kibota, T. T. & Lynch, M. Estimate of the genomic mutation rate deleterious to overall fitness in *E. coli*. *Nature* **381,** 694–696 (1996).
23. *Biolog ES Microplate Instructions for Use* (Biolog, Hayward, California, 1993).
24. Guckert, J. B. *et al.* Community analysis by Biolog: curve integration for statistical analysis of activated sludge microbial habitats. *J. Microb. Meth.* **27,** 183–197 (1996).

Acknowledgements

We thank L. Ekunwe for assistance; J. Conner, J. Cooper, N. Cooper, D. Futuyma, D. Hall, A. Jarosz, T. Marsh, P. Moore, S. Remold and D. Rozen for discussions; and M. Blot, D. Schneider, P. Sniegowski and V. Souza for sharing unpublished data. This research was supported by NSF grants to V.S.C. and R.E.L. and by the Center for Microbial Ecology.

Correspondence and requests for materials should be addressed to V.S.C. (e-mail: cooperva@msu.edu).

Natural selection and sympatric divergence in the apple maggot *Rhagoletis pomonella*

Kenneth E. Filchak, Joseph B. Roethele & Jeffrey L. Feder

Department of Biological Sciences, Galvin Life Science Center, University of Notre Dame, Notre Dame, Indiana 46556-0169, USA

In *On the Origin of Species*, Darwin proposed that natural selection had a fundamental role in speciation[1]. But this view receded during the Modern Synthesis when allopatric (geographic) models of speciation were integrated with genetic studies of hybrid sterility and inviability[2,3]. The sympatric hypothesis posits that ecological specialization after a host shift can result in speciation in the absence of complete geographic isolation[4,5]. The apple maggot, *Rhagoletis pomonella*, is a model for sympatric speciation in progress[4,5]. Hawthorn (*Crataegus* spp.) is the native host for *R. pomonella* in N. America[5]. But in the mid-1800s, a new population formed on introduced, domesticated apple (*Malus pumila*)[4,5]. Recent studies[6–10] have conferred 'host race' status on apple flies as a potentially incipient species, partially isolated from haw flies owing to host-related adaptation. However, the source of selection that differentiates apple and haw flies is unresolved. Here we document a gene–environment interaction (fitness trade-off) that is related to host phenology and that genetically differentiates the races.

Because *Rhagoletis* flies mate exclusively on or near the fruit of their host plants[11], differences in host preference can result in virtually complete premating isolation among species[12]. However, mark-release experiments have shown that such 'host fidelity' only partly restricts gene flow between apple and haw races to about 6% per year[9] (*R. pomonella* is univoltine). Despite this exchange, the races are not fusing; allozyme loci mapping to three different regions of the fly's genome display consistent allele frequency differences over time[10] (see Methods). Therefore, some form of strong host-dependent selection must be acting on these allozymes (or linked genes).

Evidence suggests that the interplay between host phenology, temperature and diapause is responsible for differentiating the

races. Field data from Grant, Michigan (MI), USA, show that the earlier ripening time of sweeter apple varieties favourable for larval survival advances the seasonal distribution of apple flies by an average of 3–4 weeks before haw flies (Fig. 1). As a result, developing apple-fly larvae and pupae experience warmer temperatures and a longer time period before winter than haw flies. For example, daily internal fruit temperatures recorded at Grant in 1999 were on average 4 °C higher in apples (mean ± s.e., 24.1 ± 0.79 °C, 20–26 August) than haws (20.1 ± 0.46 °C, 13–17 September). (Note that these data probably underestimate conditions for larvae by 1–2 °C, as they were recorded during periods just after peak larval emergence from, rather than feeding within, fruits; Fig. 1.) In addition, *Rhagoletis* overwinters in a facultative pupal diapause; pre-imago flies will forgo a prolonged diapause and eclose within 30 days if exposed to elevated temperature[10]. In nature, such 'non-diapausing' flies are doomed because they either eclose as a second generation in the late fall when no fruit is present, or break diapause prematurely in the winter, depleting vital energy reserves and starving to death. Because allozyme alleles more common to apple flies at Grant, MI, all correlate with delayed adult eclosion and recalcitrance to non-diapause development[10], we proposed that warmer conditions associated with the earlier phenology of apples select for a more muted developmental response (deeper diapause) in the apple than haw race.

To test our hypothesis, we reared haw-origin larvae and pupae under different combinations of prewinter temperature and winter length. We then scored eclosing adults for four allozymes displaying host-related frequency differences (*Dia-2*, *Me*, *Acon-2*, *Had*) and a control (*Idh*) that does not[7,8]. Our prediction was that selection should favour 'apple race' alleles segregating in the haw-fly sample under the warmer prewinter temperature simulating apple (26 °C) and 'haw race' alleles under cooler, more haw-like conditions (17 and 22 °C). Longer winters were also expected to select against 'haw' alleles because pupae with these genes would tend to terminate diapause prematurely and die during extended chilling.

Results for the 26 °C treatment matched predictions. Except for *Dia-2*, frequencies of haw alleles all significantly fell with winter length (Fig. 2). These findings paralleled a previous study in which lengthening the 26 °C prewinter period also selected against haw genes[10]. In contrast, frequencies of haw alleles in the 17 and 22 °C treatments increased with winter length (Fig. 2). Genetic response curves therefore unexpectedly crossed between prewinter treatments, revealing a complex gene–environment interaction.

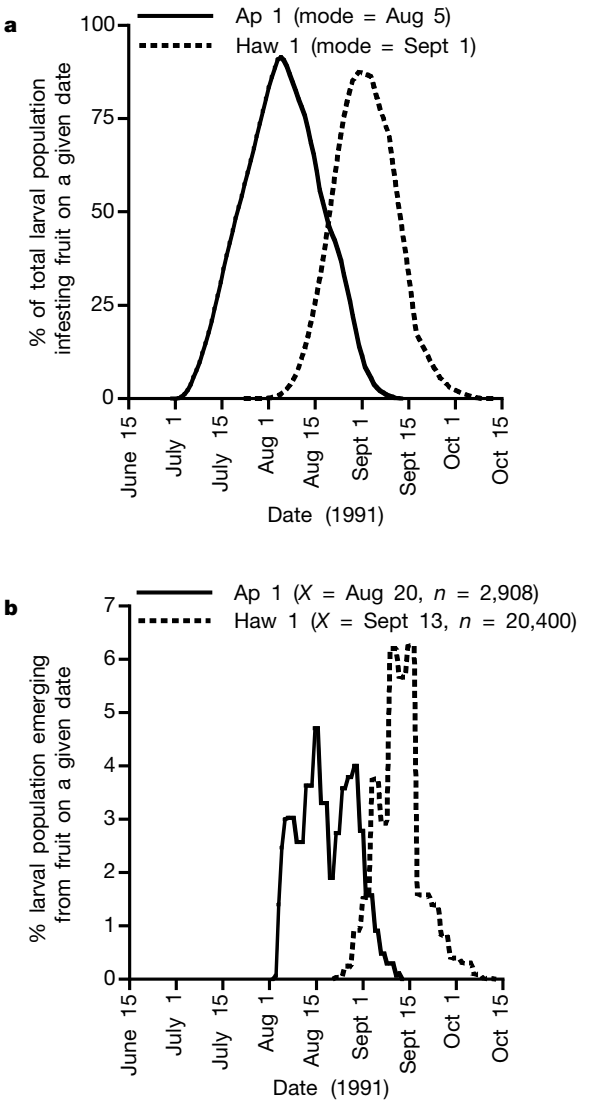

Figure 1 Seasonal distributions of fly larvae. Larvae feeding within (**a**), and emerging from (**b**), apple tree 1 & haw tree 1 fruit at Grant, MI, in 1991. (Note that these two trees support most of the fly population at Grant.) Details concerning the methods used to determine larval emergence/pupation dates can be found in experiments I–III of ref. 25. Larval feeding curves were generated by subtracting estimated development times for apple and haw flies (that is, the number of days from egg hatch in fruit to completion of larval feeding and pupation in the soil) from the distributions of larvae exiting apple and haw fruit in **b**. *X* is the mean. Methods for determining development rates in 1991 were similar to those for the 'bag' experiment in ref. 26. For a complete description of the *R. pomonella* life-cycle, see Supplementary Information.

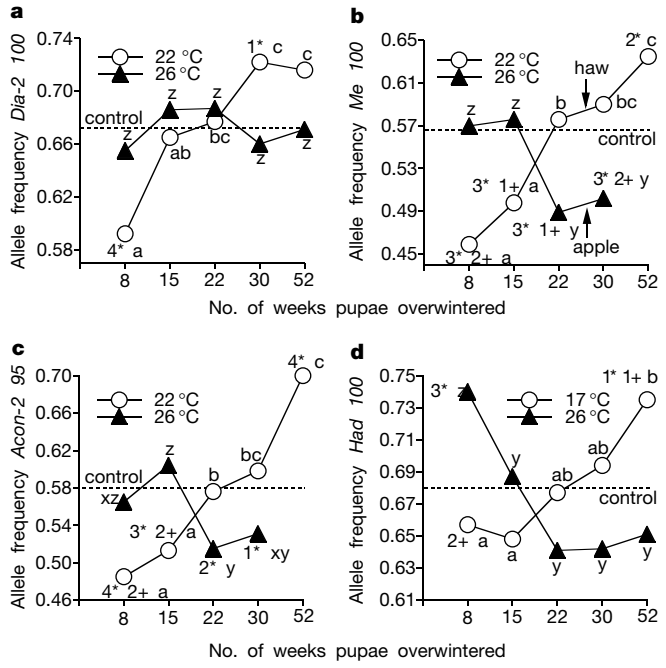

Figure 2 Comparisons of allele frequencies in eclosing adults. **a**, *Dia-2 100*; **b**, *Me 100*; and **c**, *Acon-2 100* between 26 and 22 °C treatments. **d**, *Had 100* between 26 and 17 °C treatments. Survivorship data, as well as genetic results for temperature treatments not shown and for the control locus *Idh*, are provided in Figs 2–4 of the Supplementary Information. Dashed lines are control allele frequencies. Asterisk indicates significant frequency difference between indicated sample and the control; '+' indicates significant difference between prewinter treatments for a given winter length. Numerical prefix indicates significance level (1, $P \leq 0.05$; 2, $P \leq 0.01$; 3, $P \leq 0.001$; 4, $P \leq 0.0001$; Fisher exact tests). Samples within a prewinter treatment not sharing a common letter differ significantly in allele frequency. Arrows in **b** denote response at *Me 100* for conditions best approximating those for apple and haw flies in nature.

Figure 3 depicts this interaction for *Me*, showing that absolute fitnesses for genotypes were negatively correlated across the 8- and 30-week winter periods within both the 26 °C ($r = -0.36$) and 22 °C ($r = -0.68$) treatments, indicative of fitness trade-offs. But the slopes of reaction norms had opposite signs, being negative with increased winter length for *Me 100* containing genotypes at 26 °C, and positive at 22 °C. Hence, the trade-offs result from a three-way interaction of gene, prewinter temperature and winter length, which implies that there is a threshold in the thermostatic regulation of diapause. Flies possessing haw race alleles that experience cooler temperatures (≤ 22 °C) before winter appear to set their diapause clocks to withstand long periods of chilling. However, warm temperatures (26 °C) cause these same genotypes to enter shallower diapauses, making them vulnerable to long winters. The control locus *Idh* did not respond to variation in either prewinter temperature or winter length.

The fitness trade-off can account for the genetic differentiation of the host races. Field data suggest that the 26 and 22 °C prewinter/30-week winter treatments approximate conditions faced by apple and haw flies, respectively, at our Michigan sites. (Soil temperatures at Grant in the winter of 1998–1999, as well as mean ambient recordings for Grand Rapids, MI, were below the *R. pomonella* developmental threshold[13] of 8.7 °C for about 27 weeks from 15 October to 21 April.) Given the 30-week fitness values shown in Fig. 3, computer simulations indicate that *Me 100* frequencies would equilibrate at 0.18 in the apple and 0.64 in the haw race. The predicted difference of 0.46 between the races is greater than the actual difference of 0.20 ± 0.03 at Grant from 1984–1994. Comparable results were seen for predicted (p) and observed (o) differences for *Dia-2 100* ($p = 0.14$, $o = 0.14$), *Acon-2 95* ($p = 0.28$, $o = 0.26$) and *Had 100* ($p = 0.43$, $o = 0.04$). Details concerning how prewinter length and temperature (both daily fluctuations and means) interact to affect the genetic/physiological regulation of diapause are still needed to refine our model. We must also establish whether the allozymes themselves, or linked loci, are the targets of selection. However, allozyme surveys of natural populations indicate that the races are genetically tracking variation in temperature both temporally at sites and geographically with latitude, as predicted by our results[10].

In conclusion, fitness trade-offs acting at the individual gene level are central to most models of sympatric speciation[4]. Unless the same alleles benefiting an insect on one plant are detrimental on others, gene flow and recombination could produce a 'jack-of-all-trades' genotype with high fitness on all hosts, eliminating the possibility of sympatric divergence. Although reciprocal transplants have indicated that plants often act as different selective environments for insects[14,15] and that host specialization can evolve quickly, it is unclear whether 'fundamental' trade-offs below the whole genome (organism) level were involved[16]. Here we show how divergent selection related to host phenology acts on host races of *R. pomonella*. Moreover, we demonstrate host-dependent trade-offs for the first time at the gene (genomic region) level, confirming a principal tenet of sympatric speciation. Notably, the observed allozyme response of the experimental haw population in both directions implies that considerable genetic variation exists within the races to respond to vagaries in local conditions, as well as to explore new plants with differing phenologies, potentially generating new host races and species. Other proposed cases of sympatric speciation in insects also appear to involve host/prey phenology[17–19], suggesting that host attributes affecting life-history timing may be as important as chemistry in influencing insect diet breadth and divergence. While we focused on the issue of sympatry, the message of a more direct role for natural selection and the environment in speciation applies regardless of geography. Indeed, conditions for ecological specialization are less restrictive in allopatry, due to relaxation of the constraint for fitness trade-offs[20,21]. Moreover, the limited evidence for co-speciation between insects and plants[22] suggests that adaptive radiation associated with host shifting is an important contributor to the great diversity of phytophagous insects. God's inordinate fondness for beetles may therefore reflect a more general fetish for phytophagous insects and their host plants. □

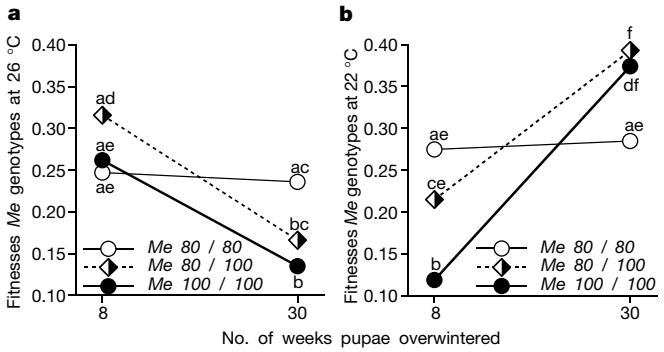

Figure 3 Absolute fitness estimates for *Me 100* genotypes related to winter length. **a**, 26 °C prewinter treatment; **b**, 22 °C prewinter treatment. Values were calculated by dividing raw genotype numbers surviving a given treatment by the expected numbers present at the start of the experiment based on frequencies in the untreated control. Estimates not sharing a common letter within or between the figures are significantly different, as determined from comparisons of 95% confidence intervals calculated assuming that survivorship follows a binomial distribution.

Methods

Fly collection and rearing

Larval-infested fruit were collected from a haw tree in E. Lansing, MI, on 5 September, 1997. Fruit were divided into three samples and held at 17, 22 or 26 °C in incubators (14/10 h light/dark cycle). Puparia were collected daily from each incubator and divided into six subsamples. One subsample was immediately frozen as a genetic control. The remaining pupae were put into petri-dishes containing moist vermiculite, returned to the incubators for 10 days, then kept at 14 °C for 2 days, before being placed in a refrigerator cycling between 0 and 5 °C to simulate winter. Dishes were removed from the cold after 8, 15, 22, 30 or 52 weeks, put in an incubator at 21 °C, and monitored for eclosing (surviving) adults over a 5-month period. Initial sample sizes were 1,352, 1,494 and 1,530 pupae for each winter length in the 17, 22 and 26 °C treatments, respectively.

Genetic analysis of flies

Standard starch gel techniques[7,8] were used to score adults for NADH-diaphorase-2 (*Dia-2*), malic enzyme (*Me*), aconitase-2 (*Acon-2*) and hydroxyacid dehydrogenase (*Had*). These four allozymes display consistent allele frequency differences between apple (a) and haw (h) races at Grant, MI (mean frequencies 1984–1994: *Dia-2 100*, $a = 0.68 \pm 0.01$, $h = 0.82 \pm 0.01$; *Me 100*, $a = 0.42 \pm 0.02$, $h = 0.62 \pm 0.02$; *Acon-2 95*, $a = 0.20 \pm 0.01$, $h = 0.46 \pm 0.01$; *Had 100* $a = 0.79 \pm 0.01$, $h = 0.83 \pm 0.01$). *Dia-2* (chromosome / linkage group 1), *Me* (linkage group 2), *Acon-2* (linkage group 2) and *Had* (linkage group 3) map to three different regions of the *R. pomonella* genome[23]. We also scored adults for a control locus, isocitrate dehydrogenase (*Idh*), that does not show host-related differentiation[7,8]. Numbers of adults scored for the 8–52-week winter treatments were 277, 310, 345, 377 and 184 for 17 °C; 205, 271, 311, 271 and 250 for 22 °C; and 334, 268, 195, 225 and 129 for 26 °C. We do not have data for *Me* and *Acon-2* for the 26 °C, 52-week sample because of technical difficulties. Results for untreated control samples were combined because no locus displayed significant heterogeneity among temperature treatments (total $n = 1,270$). We have sequenced an anonymous complementary DNA clone near *Dia-2*, *Me*, *Acon-2* and *Had* in linkage groups 1–3, and found two principal haplotypes segregating for each cDNA locus (our own unpublished data). These cDNA haplotypes were in strong gametic disequilibrium with the major 'apple' and 'haw' race electromorphs (alleles) segregating at the linked allozyme(s). The allozymes therefore reflect the allelic state of linked genes (genomic regions) and do not mask appreciable hidden genetic variation.

Temperature measurements

Internal fruit temperature was measured at Grant in 1999 using Hobo data loggers (catalogue no. H08-008-04; Onset Corp., Pocasset, MA). Thermal probes (TMC6-HC) were inserted into picked, ripe apples ($n = 4$, probe depth = 1.5 cm) and haws ($n = 4$, probe depth = 0.5 cm) placed in mottled sunlight, under the canopy on the west side of a haw tree, with temperature readings recorded automatically every 15 min. Winter length was estimated by measuring soil temperature at Grant from 1998–1999, as well as from ambient temperature data obtained over a 34-year period (1963–1996) from the national

weather station at Grand Rapids, MI, located 35 km south of Grant. Soil measurements were made using Hobo data loggers (H08-031-08) fitted with probes (H08-031-08) buried 1.5 cm in the soil, the mean depth that *Rhagoletis* pupae overwinter[24].

Computer modelling of selection

Computer simulations were conducted using a discrete generation model mirroring the univoltine life-cycle of *Rhagoletis*. A random sample of 6% of the adult population eclosing under apple and haw trees was assumed to move to the alternate host each generation, matching gene flow estimates from mark-recapture studies[9]. Hard selection was invoked by weighting the proportions of immigrants and residents on a host by the mean fitness of the emigrant and resident race, respectively. After migration, adults randomly mated within host demes, with offspring experiencing viability selection based on absolute fitness values derived from the 26 °C (apple) or 22 °C (haw) prewinter, 30-week winter treatments. Given these values, the simulations converged on a stable equilibrium for each allozyme that maintained polymorphism and host-related differentiation regardless of initial allele frequencies.

Received 24 March; accepted 31 July 2000.

1. Darwin, C. *The Origin of Species by Means of Natural Selection* (Murray, London, 1859).
2. Dobzhansky, T. *Genetics and the Origins of Species* (Columbia Univ. Press, New York, 1937).
3. Mayr, E. *Systematics and the Origin of Species* (Columbia Univ. Press, New York, 1942).
4. Bush, G. L. in *Evolutionary Strategies of Parasitic Insects and Mites* (ed. Price, P. W.) 187–206 (Plenum, New York, 1975).
5. Bush, G. L. *The Taxonomy, Cytology and Evolution of the Genus Rhagoletis in North America* (Museum of Comparative Zoology, Cambridge, Massachusetts, 1966).
6. Prokopy, R. J., Diehl, S. R. & Cooley, S. S. Behavioral evidence for host races in Rhagoletis pomonella flies. *Oecologia* **76,** 138–147 (1988).
7. Feder, J. L., Chilcote, C. A. & Bush, G. L. Genetic differentiation between sympatric host races of *Rhagoletis pomonella*. *Nature* **336,** 61–64 (1988).
8. McPheron, B. A., Smith, D. C. & Berlocher, S. H. Genetic differences between *Rhagoletis pomonella* host races. *Nature* **336,** 64–66 (1988).
9. Feder, J. L. *et al.* Host fidelity is an effective pre-mating barrier between sympatric races of the apple maggot fly. *Proc. Natl Acad. Sci. USA* **91,** 7990–7994 (1994).
10. Feder, J. L. & Filchak, K. E. It's about time: The evidence for host plant-mediated selection in the apple maggot fly, *Rhagoletis pomonella*, and its implications for fitness trade-offs in phytophagous insects. *Ent. Exp. Appl.* **91,** 211–225 (1999).
11. Prokopy, R. J., Bennett, E. W. & Bush, G. L. Mating behavior in *Rhagoletis pomonella* (Diptera: Tephritidae). I. Site of assembly. *Canad. Ent.* **103,** 1405–1409 (1971).
12. Feder, J. L. & Bush, G. L. A field test of differential host plant usage between two sibling species *Rhagoletis pomonella* fruit flies (Diptera: Tephritidae) and its consequences for sympatric models of speciation. *Evolution* **43,** 1813–1819 (1989).
13. Reid, J. A. & Laing, J. E. Development threshold and degree-days to adult emergence for overwintering pupae of the apple maggot, *Rhagoletis pomonella* (Walsh) collected in Ontario. *Proc. Ent. Soc. Ontario* **197,** 19–22 (1976).
14. Via, S. in *Ecological Genetics* (ed. Real, L. A.) 58–85 (Princeton Univ. Press, Princeton, New Jersey, 1994).
15. Craig, T. P., Horner, J. D. & Itami, J. K. Hybridization studies on the host races of *Eurosta solidaginis*: Implications for sympatric speciation. *Evolution* **51,** 1552–1560 (1997).
16. Thompson, J. N. *The Coevolutionary Process* (Univ. Chicago Press, Chicago, 1994).
17. Abrahamson, W. G. *et al.* in *Gall-Forming Insects* (eds Price, P., Mattson, W. & Baranchilov, Y.) 208–222 (USDA Forest Service Tech. Report NC-174, St. Paul, Minnesota, 1994).
18. Wood, T. K. & Keese, M. C. Host-plant-induced assortative mating in Echenopa treehoppers. *Evolution* **44,** 619–628 (1990).
19. Tauber, C. A. & Tauber, M. J. in *Speciation and Its Consequences* (eds Otte, D. & Endler, J. A.) 307–344 (Sinauer, Sunderland, Massachusetts, 1989).
20. Fry, J. D. The evolution of host specialization: are trade-offs overrated? *Am. Nat.* **148,** S84–S107 (1996).
21. Feder, J. L. in *Endless Forms: Species and Speciation* (eds Howard, D. & Berlocher, S. H.) 130–144 (Oxford Univ. Press, New York, 1998).
22. Farrell, B. D. "Inordinate fondness" explained: Why are there so many beetles? *Science* **281,** 555–558 (1998).
23. Roethele, J. B. *et al.* Towards a molecular genetic linkage map for the apple maggot fly, *Rhagoletis pomonella* (Diptera:Tephritidae): a comparison of alternative strategies. *Ann. Entomol. Soc. Am.* **90,** 470–479 (1997).
24. Lathrop, F. H. & Nickels, C. B. *The biology and control of the blueberry maggot in Washington County Maine* (Tech. Bull. 275 US Dept of Agriculture, Washington DC, 1932).
25. Feder, J. L. The effects of parasitoids on sympatric host races of the apple Maggot fly, *Rhagoletis pomonella* (Diptera: Tephritidae). *Ecology* **76,** 801–813 (1995).
26. Filchak, K. E. *et al.* A field test for host-plant dependent selection on larvae of the apple maggot fly, *Rhagoletis pomonella*. *Evolution* **53,** 187–220 (1999).

Supplementary information is available on *Nature*'s World-Wide Web site (http://www.nature.com) or as paper copy from the London editorial office of *Nature*.

Acknowledgements

We thank S. Berlocher, J. Carbol, H. Dambroski, G. Dwyer, V. Gavrilovic, D. Gibbons, D. Lodge, B. Perry, D. Prokrym, U. Stolz, S. Via and the USDA lab at Niles, Michigan. This work was supported by grants from NSF (Young investigators, Integrative Research Challenges & GRT) and the USDA to J.L.F and the University of Notre Dame.

Correspondence and requests for materials should be addressed to K.E.F. (e-mail: Filchak.1@nd.edu).

Learning of action through adaptive combination of motor primitives

Kurt A. Thoroughman*† & Reza Shadmehr*

** Department of Biomedical Engineering, Johns Hopkins University, 419 Traylor Building, 720 Rutland Avenue, Baltimore, Maryland 21205, USA*

Understanding how the brain constructs movements remains a fundamental challenge in neuroscience. The brain may control complex movements through flexible combination of motor primitives[1], where each primitive is an element of computation in the sensorimotor map that transforms desired limb trajectories into motor commands. Theoretical studies have shown that a system's ability to learn action depends on the shape of its primitives[2]. Using a time-series analysis of error patterns, here we show that humans learn the dynamics of reaching movements through a flexible combination of primitives that have gaussian-like tuning functions encoding hand velocity. The wide tuning of the inferred primitives predicts limitations on the brain's ability to represent viscous dynamics. We find close agreement between the predicted limitations and the subjects' adaptation to new force fields. The mathematical properties of the derived primitives resemble the tuning curves of Purkinje cells in the cerebellum. The activity of these cells may encode primitives that underlie the learning of dynamics.

Studies of reaching movements have demonstrated that humans construct motor commands based on a prediction of forces that will be experienced in the upcoming movement[3]. When new forces are imposed on the arm, the prediction is in error and the arm does not follow the desired trajectory[3,4]. With practice the motor commands are modified[5] and the trajectory approximates the desired path. The learning of dynamics, however, affects movements outside the region of training[3,6–8], suggesting that the brain builds a state-dependent approximation of external forces[9], called an internal model. Occasional movements with unexpectedly altered dynamics, termed 'catch trials', have been used to quantify how the internal model generalizes[3,4]. Catch trials, however, not only test the internal model for a given movement but cause errors that in turn change the internal model and affect future movements. We demonstrate that the effect of errors experienced in a given movement on subsequent movements can reveal characteristics of primitives with which motor commands are generated.

We consider the internal model to be a sensorimotor map transforming desired arm trajectories into muscle forces[10–12] through a flexible combination of a set of primitives:

$$\hat{\mathbf{f}} = W^T \mathbf{g}(\mathbf{x}^\star, \dot{\mathbf{x}}^\star, \ddot{\mathbf{x}}^\star) \qquad (1)$$

where T is the transpose operator, $\hat{\mathbf{f}}$ is a vector approximation of forces $\mathbf{f}$ to be produced by muscles to compensate for task dynamics, and $\mathbf{g}$ is a vector of scalar-valued primitives $[g_1,\ldots,g_j]^T$. Although in general $\mathbf{g}$ can depend on desired position, velocity and acceleration $(\mathbf{x}^\star, \dot{\mathbf{x}}^\star, \ddot{\mathbf{x}}^\star)$, here we investigated learning of viscous forces and therefore considered a simpler subset of primitive functions that depended only on desired velocity. The internal model is learned through experience-dependent modification of the weight matrix W. Assuming a learning rule that minimizes $\tilde{\mathbf{f}}^2 \equiv |\mathbf{f} - \hat{\mathbf{f}}|^2$, W is adjusted after a movement (indexed 1) according to:

$$\Delta W_1 = -\eta \mathbf{g}(\dot{\mathbf{x}}_1^\star)\tilde{\mathbf{f}}^T \qquad (2)$$

where η is a constant learning step. This adaptation changes the

† Present address: Volen Center of Complex Systems and Department of Biology, Brandeis University, Waltham, Massachusetts 02454, USA.