Michx.[28]. The FACE system was constructed in 1997, and has been used to fumigate (1998–2001) developing forest stands. The experiment consists of 12, 30-m-diameter FACE rings, assigned to factorial treatments of atmospheric CO_2 (ambient and 560 $\mu l\,l^{-1}$ during daytime hours) and O_3 (ambient and 46.4 $\mu l\,l^{-1}$ to 55.5 $\mu l\,l^{-1}$ during the growing season (1998–2001) in the daytime (07:00–18:59) on average for the six O_3 treatment rings). The four treatments are arranged in a randomized complete block design, with three replications of each treatment. Each FACE ring is divided by a walkway system into three parts. In one half of each ring, we planted five trembling aspen genotypes of differing O_3 and CO_2 responsiveness. The other half of each ring is further divided into two quarters; one is planted with aspen and sugar maple, *Acer saccharum* Marsh., and the other is planted with aspen and paper birch, *Betula papyrifera* Marsh.; each FACE ring is planted at a 1 × 1 m spacing. In June 1997, 12,000 trees were planted inside the rings (1,000 trees per ring). The performance of our FACE exposure system, the growth responses of the trees and additional details of our experiment have been summarized in detail elsewhere[28].

Tree height and diameter measurement

Tree height and diameter were measured at the end of each growing season, after leaf fall. Tree height was assessed using a telescoping height pole and diameter was assessed at 3 cm above the soil surface along two axes, using callipers[15].

Plant assay methods

Cuticular waxes were recovered by chloroform washing from physiologically similar, fully expanded main lateral leaves collected from aspen trees growing within each ring. Wax amount (10 ± μg) was expressed in relation to leaf surface area and quantitative leaf cuticular wax chemical composition was determined using gas chromatography (GC) and GC-mass spectrometry (MS) as described elsewhere[29].

Positive identification of *Melampsora medusae* Thuem. F. sp. *tremuloidae* was confirmed by observations of urediniospores using scanning electron microscopy (SEM) and/or light microscopy. Rust incidence was assessed in late September as percentage of infected leaves per tree and severity of rust occurrence per leaf. Their product was calculated as the incidence of infection[10].

Invertebrate surveys

Leaves used for phenolic glycosides determinations were collected during the fourth larval instar of forest tent caterpillar development. Foliage (2–3 g fresh mass) was excised at the petiole from each tree, flash frozen in liquid nitrogen, freeze-dried, ground, and stored at −20 °C before analysis. Levels of the phenolic glycosides salicortin and tremulacin were measured using high-performance thin-layer chromatography (HPTLC), with purified aspen salicortin and tremulacin as reference standards[30]. Forest tent caterpillar larvae were reared communally in large mesh bags until the fourth instar, at which time they were placed in individual mesh bags (five larvae per tree, three trees per ring) and reared to pupation.

Total numbers of aphids and natural enemies on seven aspen trees from each ring were counted. Data were $\log_{10}(n + 1)$ transformed before analysis. No winged aphids were found on any of the trees; thus all aphids are likely to have been born and developed from the initial colonization of the trees by winged females early in the growing season. The natural enemies were mainly predators (such as Coleoptera and Neuroptera) and parasitic Hymenoptera. Although the data are likely to have underestimated the total number of natural enemies (particularly winged species) exploiting the aphid populations, they represent a snapshot of the population at the time of the survey. More comprehensive sampling (for example, by using insect traps) would have included natural enemies exploiting insects feeding on all the trees growing in the FACE rings, rather than aspen-feeding aphids. The long-term aphid and natural enemy population surveys used similar methods, except that data were collected from the lowest south-facing branch on each tree rather than from entire trees. In the previous survey, aspen aphids were found only on the lower branches; no aphids were ever found on the upper branches.

Statistical considerations

Data were analysed by general linear models analyses of variance (ANOVA). The Aspen-FACE experimental design, at the whole-plot (error d.f. = 6) level, is three replications of a randomized complete block design with four treatment combinations. We assume that replications are random effects, and treatments are fixed effects[28].

Received 17 April; accepted 11 July 2002; doi:10.1038/nature01028.

1. IPCC *Climate Change 2001: The Scientific Basis* (Report of Working Group I of the Intergovernmental Panel on Climate Change, IPCC Secretariat, Geneva, 2001); available at http://www.ipcc.ch/pub/spm22-01.pdf.
2. Ciais, P., Peylin, P. & Bousquet, P. Regional biospheric fluxes as inferred from atmospheric CO_2 measurements. *Ecol. Appl.* **10,** 1574–1589 (2000).
3. Fowler, D. *et al.* The global exposure of forests to air pollutants. *Wat. Air Soil Pollut.* **116,** 5–32 (1999).
4. McLaughlin, S. B. & Percy, K. E. Forest health in North America: Some perspectives on actual and potential roles of climate and air pollution. *Wat. Air Soil Pollut.* **116,** 151–197 (1999).
5. Perala, D. A. *Silvics of North America* Hardwoods (eds Burns, R. M. & Honkala, B. H.) **Vol. 2 555–569 (USDA Forest Service,** Washington, DC, 1990).
6. Barnola, J. M. *et al.* CO_2 evolution during the last millennium as recorded by Antarctic and Greenland ice. *Tellus B* **47,** 264–272 (1995).
7. Finlayson-Pitts, B. J. & Pitts, J. N. Jr *Chemistry of the Upper and Lower Atmosphere* (Academic, San Diego, CA, 1999).
8. Dickson, R. E. *et al.* Growth of five hybrid poplar genotypes exposed to interacting elevated CO_2 and O_3. *Can. J. Forest Res.* **28,** 1706–1716 (1998).
9. Awmack, C. S. & Leather, S. R. Host plant quality and fecundity in herbivorous insects. *Annu. Rev. Entomol.* **47,** 817–844 (2002).
10. Karnosky, D. F. *et al.* Interacting CO_2, tropospheric O_3 and predisposition of aspen *Populus tremuloides* Michx. to attack by *Melampsora medusae* rust. *Glob. Change Biol.* **8,** 329–338 (2002).
11. Hendrey, G. R., Ellsworth, D. S., Lewin, K. F. & Nagy, J. A free-air system for exposing tall forest vegetation to elevated atmospheric CO_2. *Glob. Change Biol.* **5,** 293–309 (1999).
12. Ziller, W. G. *The Tree Rusts of Western Canada* (Canadian Forestry Service Publication No. 1329, Victoria, British Columbia, 1974).
13. Martineau, R. *Insects Harmful to Forest Trees* (Agriculture Canada, Canadian Forest Service, Quebec, 1984).
14. USDA Forest Service *Aphids* (Forest Health Protection, Southern Region); available at http://fhpr8.srs.fs.fed.us/idotis/insects/aphids.html (2001).
15. Isebrands, J. G. *et al.* Growth responses of *Populus tremuloides* clones to interacting carbon dioxide and tropospheric ozone. *Environ. Pollut.* **115,** 359–371 (2001).
16. David, A. J., Zasada, J. C., Gilmore, D. W. & Landäusser, S. M. Current trends in the management of aspen and mixed aspen forests for sustainable production. *Forest Chron.* **77,** 525–532 (2001).
17. Jeffree, C. E. *Plant Cuticles: An Integrated Functional Approach* (ed. Kerstiens, G.) 33–82 (Bios Scientific, Oxford, 1996).
18. Eigenbrode, S. D. *Plant Cuticles: An Integrated Functional Approach* (ed. Kerstiens, G.) 201–221 (Bios Scientific, Oxford, 1996).
19. Lindroth, R. L. & Hwang, S.-Y. Diversity, redundancy and multiplicity in chemical defense system of aspen. *Rec. Adv. Phytochem.* **30,** 25–56 (1996).
20. Mendgen, K. *Plant Cuticles: An Integrated Functional Approach* (ed. Kerstiens, G.) 175–188 (Bios Scientific, Oxford, 1996).
21. Karnosky, D. F. *et al.* Effects of tropospheric O_3 on trembling aspen and interaction with CO_2: results from an O_3-gradient and a FACE experiment. *Wat. Air Soil Pollut.* **116,** 311–322 (1999).
22. Holloway, P. J. Chemistry of leaf waxes in relation to wetting. *J. Sci. Fd Agric.* **20,** 124–128 (1969).
23. Davis, C. & Meyer, T. *Field Guide to Tree Diseases of Ontario* (Natural Resources Canada, Canadian Forest Service, Sault Ste. Maire, Ontario, 1997).
24. Fitzgerald, T. D. *The Tent Caterpillars* (Cornell Univ. Press, Ithaca, New York, 1995).
25. Parry, D., Goyer, R. A. & Lenhard, G. J. Macrogeographic clines in fecundity, reproductive allocation, and offspring size of the forest tent caterpillar *Malacosoma disstria*. *Ecol. Entomol.* **26,** 281–291 (2001).
26. IPCC *Summary for Policymakers to Climate Change 2001: Synthesis Report of the Third IPCC Assessment Report* (IPCC Secretariat, Geneva, Switzerland, 2001); available at http://www.ipcc.ch/pub/SYR-text.pdf.
27. Tuskan, G. A. & Walsh, M. E. Short-rotation woody crop systems, atmospheric carbon dioxide and carbon management: A U.S. case study. *Forest. Chron.* **77,** 259–264 (2001).
28. Dickson, R. E. *et al. Forest Atmosphere Carbon Transfer Storage-II (FACTSII)—The Aspen Free-Air CO_2 and O_3 Enrichment (FACE) Project: An Overview* (USDA Forest service General Tech. Rep. NC-214, St Paul, MN, 2000).
29. Percy, K. E., McQuattie, C. J. & Rebbeck, J. A. *Air Pollutants and the Leaf Cuticle* (eds Percy, K. E., Cape, J. N., Jagels, R. & Simpson, C. M.) 67–79 (Springer, Heidelberg, 1994).
30. Lindroth, R. L., Kinney, K. K. & Platz, C. L. Responses of deciduous trees to elevated atmospheric CO_2: productivity, phytochemistry and insect performance. *Ecology* **74,** 763–777 (1993).

Acknowledgements The FACTSII: Aspen FACE project is supported by the US Department of Energy-Office of Biological and Environmental Research (DOE-BER) through its Program for Ecosystem Research. The research described herein is supported by companion grants from the US Forest Service Northern Global Change Program, Michigan Technological University, the USDA National Research Initiatives Program, the Canadian Federal Panel on Energy Research and Development (PERD), Natural Resources Canada-Canadian Forest Service, the National Science Foundation, the Natural Environmental Research Council of the United Kingdom, the British Ecological Society and the Praxair Foundation. The Aspen FACE project is an experiment within the International Biosphere Geosphere Programme and the only open-air site in the world that combines the effects of CO_2 and O_3. It is a multi-agency, interdisciplinary project with 50 scientists from seven countries.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to K.E.P. (e-mail: kpercy@nrcan.gc.ca).

Genetic mechanisms of floral trait correlations in a natural population

Jeffrey K. Conner

Kellogg Biological Station and Department of Plant Biology, Michigan State University, 3700 East Gull Lake Drive, Hickory Corners, Michigan 49060, USA

Genetic correlations among traits are important in evolution, as they can constrain evolutionary change or reflect past selection for combinations of traits[1,2]. Constraints and integration depend on whether the correlations are caused by pleiotropy or linkage disequilibrium[3], but these genetic mechanisms underlying correlations remain largely unknown in natural populations[4]. Quan-

titative trait locus (QTL) mapping studies do not adequately address the mechanisms of within-population genetic correlations because they rely on crosses between distinct species, inbred lines or selected lines (see ref. 5), and they cannot distinguish moderate linkage disequilibrium from pleiotropy because they commonly rely on only one or two episodes of recombination[6]. Here I report that after nine generations of enforced random mating (nine episodes of recombination), correlations between six floral traits in wild radish plants are unchanged, showing that pleiotropy generates the correlations. There is no evidence for linkage disequilibrium despite previous correlational selection acting on one functionally integrated pair of traits[7]. This study provides direct evidence of the genetic mechanisms underlying correlations between quantitative traits in a natural population and suggests that there may be constraints on the independent evolution of pairs of highly correlated traits.

In the past two decades, biologists have become increasingly aware of the profound effects that genetic correlations can have on evolutionary change[3,8]. A genetic correlation between two phenotypic traits can be caused either by pleiotropy, in which one locus affects both traits, or by linkage disequilibrium, in which the two traits are affected by distinct gene loci but some evolutionary force creates and maintains a nonrandom association between the alleles present at these loci[9]. Genetic correlations are important because natural selection on one character either causes an evolutionary change in a correlated neutral character or alters the response to selection in a correlated character that is itself under direct selection. In other words, non-adaptive evolution of the second character can occur. Therefore, genetic correlations among traits can slow or constrain the evolution of the most advantageous combinations of traits, at least in the short term[1,10].

But constraints will only occur if the correlation is caused by pleiotropy or extremely tight linkage; correlations caused by linkage disequilibrium between moderately or loosely linked loci can be altered rapidly by recombination and selection[4,8]. Conversely, natural selection may alter correlations, especially in cases where two or more traits interact to carry out a given function[2,11]. Here again the genetic mechanism is important, because selection is one of the principal factors that can create and maintain linkage disequilibrium[3], although selection can alter pleiotropic correlations as well. Although pleiotropy is thought to be ubiquitous and the most common mechanism of genetic correlations in nature[11–13], the limited information available from mapping studies suggests that both linkage disequilibrium and pleiotropy can be important[5,14].

I studied mechanisms of correlations among six floral traits in a natural population of wild radish, *Raphanus raphanistrum*[15]. Wild radish is self-incompatible and therefore an obligate outcrosser. Linkage disequilibrium will only occur in natural populations of

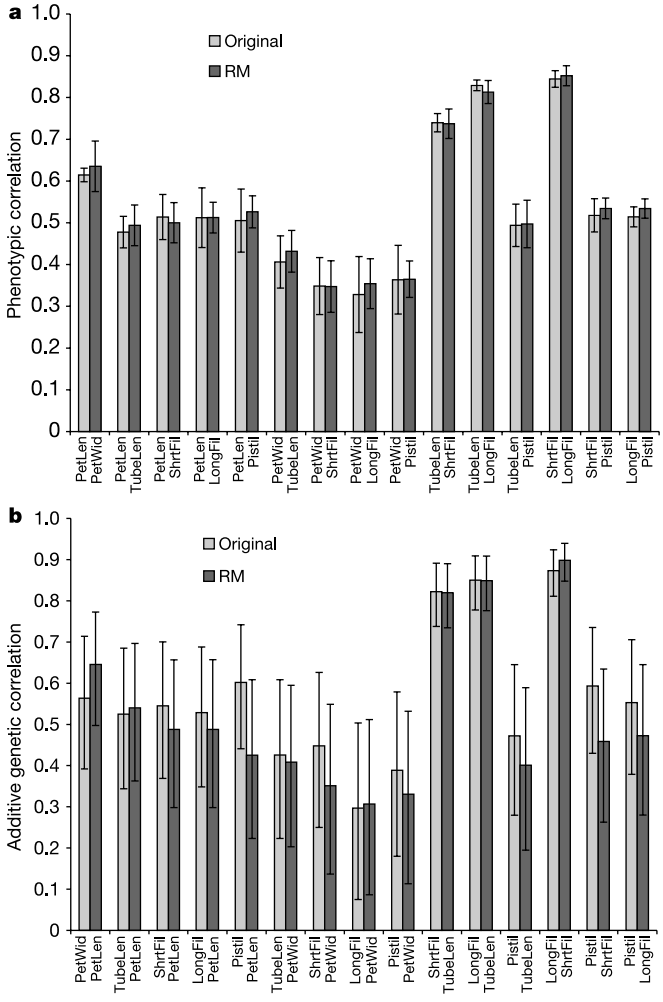

Figure 1 Floral trait correlations with 95% confidence intervals. **a**, Phenotypic correlations based on mean matrices; residual matrices gave virtually identical results (see Methods). The confidence intervals are twice the standard errors of these means. **b**, Additive genetic correlations, calculated as correlations among breeding values determined using BLUP; component correlations gave virtually identical results (see Methods). Confidence intervals are from the *z* transformation[29]. The RM data are the average of the two random-mated replicates. PetLen, petal length; PetWid, petal width; TubeLen, corolla tube length; ShrtFil, short filament length; LongFil, long filament length; Pistil, pistil length.

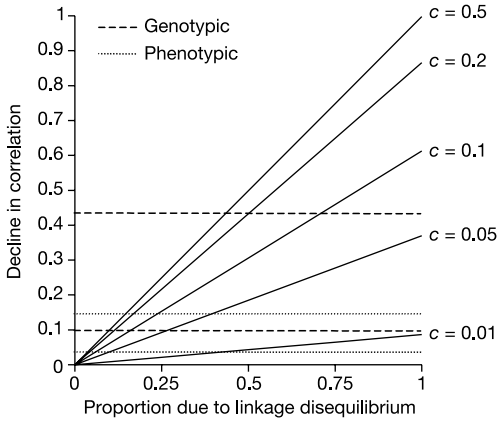

Figure 2 Theoretical declines in correlations caused by a reduction in linkage disequilibrium during nine generations of random mating. The magnitude of the reduction in a correlation is plotted on the *y* axis, the proportion of the original correlation that was due to linkage disequilibrium, that is, one minus the proportion owing to pleiotropy, is plotted on the *x* axis. Note that intermediate values for this proportion assume that several loci are affecting the correlation. The plotted lines show the declines over nine generations for five different rates of recombination from $1 - (1 - c)^9$, where c is the recombination rate[3]. For polygenic traits, c is a weighted average of the recombination rates between the various loci that affect the traits[30]. Dotted and dashed lines show the range of detectable changes in phenotypic and genetic correlations, respectively. The upper dotted and dashed lines are the detectable declines for the correlation with the largest combined 95% confidence interval (CI) in the experiment (long filament and petal width, Fig. 1); that is, with a decline of 0.15 [(original CI + RM CI)/2 = 0.15] in the phenotypic correlation or 0.43 in the genetic correlation, the 95% confidence intervals would not overlap. The lower dotted and dashed lines are the detectable declines for the correlations with the smallest confidence intervals in the experiment (the correlations between the long filament, the short filament and corolla tube lengths).

outcrossers if some force such as selection or drift counteracts recombination. The phenotypic and genetic correlation between filament and corolla tube length in wild radish is much greater in magnitude than are other floral correlations in this species[15], and there is much evidence for correlational selection acting on these traits[7,16]. Therefore, linkage disequilibrium is a plausible mechanism for at least some of the filament–corolla tube correlation. The corolla and stamens (including the filaments) have been shown to have a very close developmental relationship in many plant species[17], which might suggest that the higher correlation is due solely to pleiotropy. Although some other species in the Brassicaceae (the family to which wild radish belongs) share the very high filament–corolla tube correlation, many do not[16], indicating that a close developmental relationship may not necessarily lead to a high correlation, or that the close developmental relationship is evolutionarily labile.

I used seeds from 600 randomly selected field plants (see ref. 15 for details) to create two greenhouse populations of 300 plants each (RM1 and RM2). In each generation, crosses were carried out randomly so that every plant acted as a male and a female, but with no reciprocal crosses. One plant from each maternal plant was raised to form the next generation. In this mating design there is no selection, because all plants have equal fitness. Under such relaxed selection, recombination will reduce the contribution of linkage disequilibrium to genetic correlations, whereas the contribution of pleiotropy will persist.

My design is similar in many respects to one used to measure mutation accumulation[18]. The effects of mutation accumulation should be minimal in my study because, first, floral traits were measured rather than fitness, which means that there should be far fewer genes involved; second, the traits were measured under benign conditions, which minimizes the phenotypic effects of mutations (ref. 18 and references therein); and third, the study only lasted nine generations. The absence of mutational effects is supported by the lack of difference between the mean values of the traits in the random-mated and those of the control populations (see below).

After nine generations of random mating (that is, nine episodes of recombination), 275–298 plants were raised from each of the two randomly mated populations and from a random sample of the original field population; the latter were from seeds stored from the same field collection used to establish the random mating populations. In each group, 68–75 plants were chosen randomly to be sires, and each was used to pollinate 1–3 unique randomly chosen plants (a total of 212 sires and 601 dams). One seed from each dam was raised in the greenhouse in each of two blocks separated in time for a total of 1,172 offspring (30 offspring failed to germinate). In both parental and offspring generations, the three groups were interspersed in a systematic fashion on the greenhouse bench, eliminating any average environmental differences between the groups. Six floral dimensions (petal width and lengths of petal, corolla tube, short and long filaments, and pistil) were measured on 1 of the first 40 flowers produced by each plant in both generations (93% of these were the third to thirteenth flower; see ref. 15 for details).

Owing to the design of this study and previous work on this species, differences in the phenotypic correlations among treatment groups are likely to reflect accurately differences in the additive genetic correlations. The phenotypic analyses have greater power than have genetic analyses, because power for the former depends on all individuals measured, whereas the number of families (always a smaller number) is more crucial for the latter[19]. But it is possible that environmental or non-additive genetic variances and covariances differ among the groups in ways that would create phenotypic differences without additive genetic differences or obscure phenotypic differences even if genetic changes had occurred. For these reasons I present both additive genetic and phenotypic analyses, which are in excellent agreement with each other—there was no evidence for changes in the means, variances, covariances or correlations among traits owing to the nine generations of recombination.

Multivariate analysis of variance showed no evidence for differences in the mean values of the traits among the three treatment groups. Although there was a strong block effect ($P < 0.0001$), neither the linear contrast of the two RM populations to the original population ($F_{6,2001} = 1.6$, $P = 0.14$) or the interaction of group with block ($F_{48,6982} = 1.2$, $P = 0.20$) was significant, despite very high statistical power. This suggests that effects of selection, mutation and drift on the traits during the experiments were negligible.

The genetic variance/covariance matrices (G) did not differ significantly (likelihood ratio tests[20]: RM1 versus original, $\chi^2_{21} = 26.4$, $P = 0.19$; RM2 versus original, $\chi^2_{21} = 9.93$, $P = 0.97$; total $N = 1{,}985$). Common principal components (CPC) analysis[19] of the phenotypic variance/covariance matrices (P) also did not detect significant differences between the RM and the original matrices. Because the CPC method tends to overestimate differences between matrices[21], and the total sample was very large ($N = 2{,}021$), the lack of significant difference is strong evidence that the matrices were not altered by the random mating.

The phenotypic and genetic correlation matrices also showed no evidence for declines associated with linkage disequilibrium (Fig. 1 and Table 1): the 95% confidence intervals were broadly overlapping for all correlations, the average changes were 0.07 or less, and the matrices were very highly correlated. Note that although a few of the genetic correlations declined moderately (up to 0.18), the correlations between the lengths of the filaments and corolla tube did not.

Overall, the evidence indicates that floral correlations are caused by pleiotropy, with no evidence for linkage disequilibrium. The means, variances, covariances and correlations for the six floral traits were highly stable over nine generations. My power to detect linkage disequilibrium depends on many factors, including the proportion of the original correlation caused by linkage disequilibrium, the degree of initial disequilibrium among independent loci affecting the traits, and the weighted mean recombination rate among these loci (refs 11, 13, 22, 23 and Fig. 2). My experiment had a high probability to detect declines in phenotypic correlations (Fig. 2, dotted lines) and in genetic correlations with smaller 95% confidence intervals (Fig. 2, lower dashed line), including those between the filaments and corolla tube. For these, linkage disequilibrium would have been detected unless only a very small proportion of the correlation was caused by linkage disequilibrium or if the correlation was caused by loci with harmonic mean recombination rates in the region of 0.01. For the genetic correlations with larger confidence intervals (Fig. 2, upper dashed line denotes the largest), linkage disequilibrium would still have been detectable if more than half of the correlation was caused by linkage disequilibrium and the mean recombination rate was at least 0.2. A mean recombination rate lower than 0.2 is unlikely in wild radish—an outcrosser with nine pairs of chromosomes[13,23]—unless loci affecting the traits are tightly clustered on a small subset of these chromosomes.

These pleiotropic correlations would be expected to constrain the

Table 1 **Comparisons of phenotypic and additive genetic correlation matrices**

	Average difference	Maximum decline	Matrix correlation
Phenotypic (average)	0.008	−0.016	0.997
Phenotypic (residuals)	0.011	−0.015	0.995
Additive genetic (component)	−0.069	−0.169	0.984
Additive genetic (breeding value)	−0.040	−0.177	0.939

Comparisons were between the original and random-mated populations. Four different methods of calculating phenotypic and genetic correlation matrices were used (Methods). Absolute values were not used in calculating the average difference, as recommended previously[28], because all of the floral correlations are positive and because the direction of the change is of interest.

independent evolution of floral traits in wild radish, particularly the filaments and corolla tube for which the correlations are very high. Studies of populations in species that have diverged naturally or through artificial selection have also not generally detected changes in the *G*-matrix (refs 19, 24, and references therein; but see also ref. 22). These results also have significance for discussions of possible transient changes in the *G*-matrix caused by selection that creates linkage disequilibrium[22,23]; no evidence of such changes was found in this study. It has been argued that most estimates of the *G*-matrix are made under relaxed selection and therefore do not reflect the *G*-matrix under selection in natural populations[22]. At least in the population studied here there was no difference between the two, removing one of the many caveats involved in reconstructing past selection.

The finding of correlations that are due to pleiotropy rather than linkage disequilibrium in an obligately outcrossing species is not unexpected on theoretical grounds[11]. But this expectation is based on several assumptions, including that of weak selection; epistatic or correlational selection can create linkage disequilibrium in outcrossing species[11,25]. Because there is evidence for correlational selection on the lengths of the filaments and corolla tube in wild radish[7,16], the empirical finding of a lack of linkage disequilibrium is significant. The lack of linkage disequilibrium does not mean that selection has not altered the filament–corolla tube correlations; selection can alter correlations caused by pleiotropy as well[13]. Future studies of how correlations respond to correlational selection would improve our understanding of the evolution of integration among functionally related traits. My study provides a direct demonstration of the mechanisms underlying correlations among quantitative traits in a natural population; similar studies on additional traits and species will be crucial to furthering our knowledge of integration and constraint in evolution. □

Methods

Details of mating design

On average, 3% of mothers had no offspring that successfully produced seed in the next generation owing to failures in germination, mortality or unsuccessful pollination; thus, there may have been some selection. Some or all of the lack of germination (the single most common source of failure) was probably caused by environmental stresses on the mothers, especially insect and pathogen attacks, which were frequent in the greenhouse. Evidence for this comes from the pattern of average germination success, which showed no trend over the generations and little variance between the two replicates in a generation, but varied widely from generation to generation. If germination success or resistance or tolerance to enemies was genetically correlated with the floral traits, then there could have been some correlated evolution in the floral traits. These types of genetic correlation are largely unknown, except that there are no significant genetic correlations between germination time and any of the floral traits measured[15]. When a total failure of offspring reproduction from one maternal parent occurred, two plants were chosen randomly, one to be used twice as a male and the other twice as a female to maintain each population close to 300 individuals. Because most individuals had two offspring in the next generation, the effective population size in each population was roughly 600, and the inbreeding coefficient increased by only about 1 in 1,200 each generation[26].

Analysis details

I compared *G*-matrices using the program pcrf1, which is part of the Quercus package[20]. Restricted maximum-likelihood (REML) estimates were made with and without the pair of matrices constrained to be the same, and the fit of the models was compared using two times the difference in log-likelihood, which has a χ^2 distribution. Because the floral traits were measured on all parents and offspring, and Quercus takes advantage of all pedigree information, each of these comparisons are based on two-thirds (each RM replicate was tested against the original population separately) of the complete sample of $N = 1{,}985$ individuals.

Phenotypic variance/covariance matrices (*P*) and phenotypic correlations (Table 1 and Fig. 1) were calculated in two different ways. Both methods were designed to correct for differences in means among blocks and generations that could bias the correlation estimates if all data were simply pooled. In neither case was a significant difference detected between the *P*-matrices, that is, none of the CPC tests was significant at $P < 0.05$ on the basis of 1,000 randomizations.

In the first method, the phenotypic covariance matrices were estimated from residuals of models correcting for differences in means among generations, blocks nested within generations, replicates (for the two RM populations only) and the interactions between them. Therefore, a single test was done comparing the RM to the original populations with $N = 1{,}317$ and 704, respectively. This total *N* is slightly greater than the genetic analysis above because some of the parents did not produce flowering offspring and were therefore eliminated from the genetic analysis. In the second method, I calculated a *P*-matrix for each block and generation separately, and then averaged the corresponding elements across the five matrices for the original population (three parental and two offspring blocks) and across the ten matrices for the random mated populations (5 blocks × 2 replicates). The CPC tests were done on these averages.

The genetic correlations (Table 1 and Fig. 1) were also calculated in two ways: variance/covariance component correlations were estimated by REML in Quercus (see above), and breeding value correlations were estimated as Pearson's product–moment correlations among breeding values calculated using best linear unbiased prediction (BLUP). BLUPs were estimated using Proc Mixed in SAS[27].

Received 15 June; accepted 19 August 2002; doi:10.1038/nature01105.

1. Maynard Smith, J. *et al.* Developmental constraints and evolution. *Quart. Rev. Biol.* **60,** 265–287 (1985).
2. Deng, H.-W., Haynatzka, V., Spitze, K. & Haynatzki, G. The determination of genetic covariances and prediction of evolutionary trajectories based on a genetic correlation matrix. *Evolution* **53,** 1592–1599 (1999).
3. Lynch, M. & Walsh, B. *Genetics and Analysis of Quantitative Traits* (Sinauer Associates, Sunderland, Massachusetts, 1998).
4. Clark, A. G. in *Genetic Constraints on Adaptive Evolution* (ed. Loeschcke, V.) 25–45 (Springer, Berlin, 1987).
5. Paterson, A. H. *et al.* Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature* **335,** 721–726 (1988).
6. Kearsey, M. J. & Farquhar, A. G. L. QTL analysis in plants; where are we now? *Heredity* **80,** 137–142 (1998).
7. Morgan, M. T. & Conner, J. K. Using genetic markers to directly estimate male selection gradients. *Evolution* **55,** 272–281 (2001).
8. Futuyma, D. J. *Evolutionary Biology* (Sinauer Associates, Sunderland, Massachusetts, 1998).
9. Falconer, D. S. & Mackay, T. F. C. *Introduction to Quantitative Genetics* (Longman, Harlow, 1996).
10. Mitchell-Olds, T. Pleiotropy causes long-term genetic constraints on life-history evolution in *Brassica rapa. Evolution* **50,** 1849–1858 (1996).
11. Lande, R. The genetic correlation between characters maintained by selection, linkage and inbreeding. *Genet. Res.* **44,** 309–320 (1984).
12. Wright, S. *Evolution and the Genetics of Populations. Vol. 2. The Theory of Gene Frequencies* (Univ. Chicago Press, Chicago, 1969).
13. Lande, R. The genetic covariance between characters maintained by pleiotropic mutations. *Genetics* **94,** 203–215 (1980).
14. Nuzhdin, S. V., Dilda, C. & Mackay, F. C. The genetic architecture of selection response: Inferences from fine-scale mapping of bristle number quantitative trait loci in *Drosophila melanogaster. Genetics* **153,** 1317–1331 (1999).
15. Conner, J. K. & Via, S. Patterns of phenotypic and genetic correlations among morphological and life-history traits in wild radish, *Raphanus raphanistrum*. *Evolution* **47,** 704–711 (1993).
16. Conner, J. K. Floral evolution in wild radish: the roles of pollinators, natural selection, and genetic correlations among traits. *Int. J. Plant Sci.* **158,** S108–S120 (1997).
17. Hill, J. P. & Lord, E. M. Floral development in *Arabidopsis thaliana*: a comparison of the wild type and the homeotic pistillata mutant. *Can. J. Bot.* **67,** 2922–2936 (1989).
18. Shabalina, S. A., Yampolsky, L. Y. & Kondrashov, A. S. Rapid decline of fitness in panmictic populations of *Drosophila melanogaster* maintained under relaxed natural selection. *Proc. Natl Acad. Sci. USA* **94,** 13034–13039 (1997).
19. Phillips, P. C. & Arnold, S. J. Hierarchical comparison of genetic variance-covariance matrices. I. Using the Flury hierarchy. *Evolution* **53,** 1506–1515 (1999).
20. Shaw, R. G. The comparison of quantitative genetic parameters between populations. *Evolution* **45,** 143–151 (1991).
21. Houle, D., Mezey, J. & Galpern, P. Interpretation of the results of common principal components analysis. *Evolution* **56,** 433–440 (2002).
22. Shaw, F. H., Shaw, R. G., Wilkinson, G. S. & Turelli, M. Changes in genetic variances and covariances: G whiz! *Evolution* **49,** 1260–1267 (1995).
23. Bulmer, M. G. Linkage disequilibrium and genetic variability. *Genet. Res.* **23,** 281–289 (1974).
24. Roff, D. A. & Mousseau, T. A. Does natural selection alter genetic architecture? An evaluation of quantitative genetic variation among populations of *Allonemobius socius* and *A. fasciatus*. *J. Evol. Biol.* **12,** 361–369 (1999).
25. Whitlock, M. C., Phillips, P. C., Moore, F. B. G. & Tonsor, S. J. Multiple fitness peaks and epistasis. *Annu. Rev. Ecol. System.* **26,** 601 (1995).
26. Crow, J. F. & Kimura, M. *An Introduction to Population Genetics Theory* (Harper and Row, New York, 1970).
27. SAS Institute *SAS/STAT User's Guide, Version 8* (SAS Institute, Cary, North Carolina, 1999).
28. Willis, J. H., Coyne, J. A. & Kirkpatrick, M. Can one predict the evolution of quantitative characters without genetics? *Evolution* **45,** 441–444 (1991).
29. Snedecor, G. W. & Cochran, W. G. *Statistical Methods* (Iowa State Univ. Press, Ames, Iowa, 1989).
30. Bulmer, M. G. *The Mathematical Theory of Quantitative Genetics* (Oxford Univ. Press, Oxford, 1985).

Acknowledgements I thank C. Haun, T. Hickox, S. Kercher, J. Mudd, C. Stewart and many undergraduates for help in the greenhouse; P. Phillips for help with the CPC analysis and for programs; F. Shaw for analyses with Quercus; E. Brodie, B. Charlesworth, J. Fry, D. Houle, R. Lande, M. Lynch, D. Schluter, M. Turelli, S. Via and M. Whitlock for discussion; and A. Agrawal, M. Duffy, J. Fant, J. Kelly, M. Morgan, P. Phillips, H. Sahli, C. Stewart and S. Via for comments on the manuscript. This work was supported by the US National Science Foundation, the University of Illinois and Michigan State University.

Competing interests statement The author declares that he has no competing financial interests.

Correspondence and requests for materials should be addressed to the author (e-mail: conner@kbs.msu.edu).

