

Acknowledgements

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Correspondence and requests for materials and should be addressed to A.L.D. (e-mail: aldowin@owu.edu).

Low host specificity of herbivorous insects in a tropical forest

Vojtech Novotny*, Yves Basset†, Scott E. Miller‡, George D. Weiblen§, Birgitta Bremer||, Lukas Cizek* & Pavel Drozd¶

* Institute of Entomology, Czech Academy of Sciences and Biological Faculty, University of South Bohemia, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic

† Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Ancon, Panama

‡ Department of Systematic Biology, National Museum of Natural History, Smithsonian Institution, Washington DC 20560-0105, USA

§ Department of Plant Biology, University of Minnesota, 220 Biological Sciences Center, 1445 Gortner Avenue, St Paul, Minnesota 55108-1095, USA

|| Bergius Foundation at the Royal Swedish Academy of Sciences, PO Box 50017, SE-104 05 Stockholm, Sweden

¶ University of Ostrava, Department of Biology, 30. dubna 22, 701 03 Ostrava, Czech Republic

Two decades of research^{1–4} have not established whether tropical insect herbivores are dominated by specialists or generalists. This impedes our understanding of species coexistence in diverse rainforest communities. Host specificity and species richness of tropical insects are also key parameters in mapping global patterns of biodiversity^{1,4,5}. Here we analyse data for over 900 herbivorous species feeding on 51 plant species in New Guinea and show that most herbivorous species feed on several closely related plant species. Because species-rich genera are dominant in tropical floras, monophagous herbivores are probably rare in tropical forests. Furthermore, even between phylogenetically distant hosts, herbivore communities typically shared a third of their species. These results do not support the classical view that the coexistence of herbivorous species in the tropics is a consequence of finely divided plant resources; non-equilibrium models of tropical diversity⁶ should instead be considered. Low host specificity of tropical herbivores reduces global estimates of arthropod diversity from 31 million (ref. 1) to 4–6 million species. This finding agrees with estimates based on taxonomic collections, reconciling an order of magnitude discrepancy between extrapolations of global diversity based on ecological samples of tropical communities with those based on sampling regional faunas^{7,8}.

Host specificity is difficult to measure, and the limitations of existing studies include sampling only certain taxonomic groups rather than entire guilds, or sampling limited numbers of host plant species and lineages. Studies are often of insufficient duration, producing samples too small for quantitative analysis, or insects are sampled destructively, which precludes feeding experiments and the study of immature stages. Further, previous studies² failed to consider the phylogenetic relationships of host plants by using

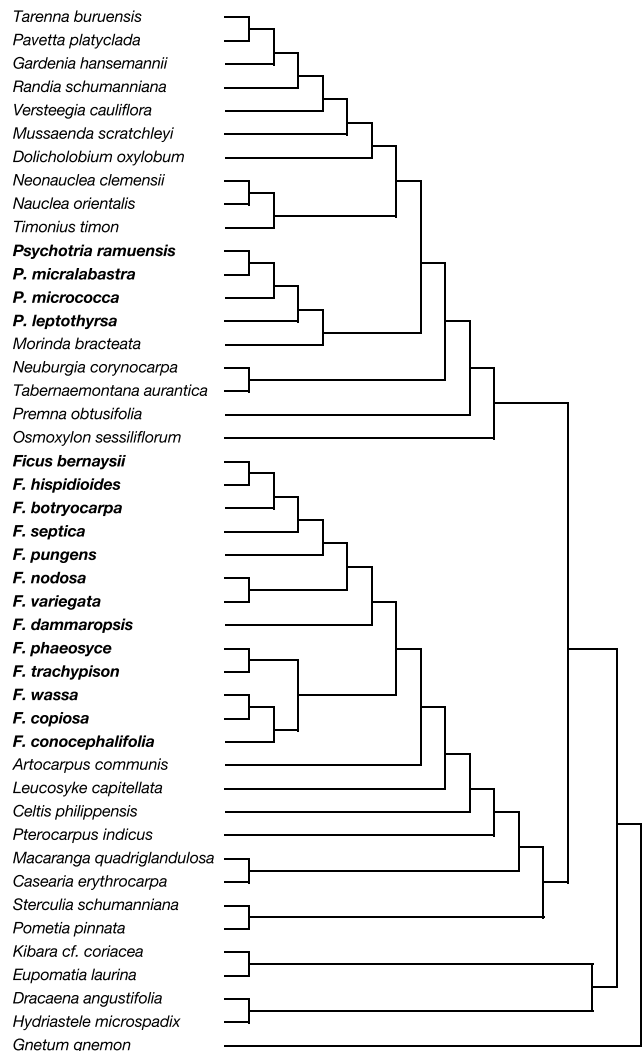


Figure 1 Phylogenetic relationships of host plants included in the study.

measures of host specificity that relied on counts of higher plant taxa (for example, genera or families). This approach can be misleading when taxonomic ranks are not commensurate with plant lineages. We examined the impacts of sampling bias and phylogenetic effects on estimates of host specificity by analysing the largest available data set of its kind. The leaf-chewing insect community on 51 plant species was characterized by using a sample

Table 1 Overlap between leaf-chewing communities from closely and distantly related host plants

Host plant	Herbivores	<i>r</i>	So (mean ± s.e.m.)
<i>Ficus</i> spp.	Coleoptera	–0.182	0.51 (±0.008)
	Lepidoptera	–0.274	0.52 (±0.010)
	Orthopteroids	–0.267	0.48 (±0.019)
	Total	–0.370	0.51 (±0.007)
			0.57 (±0.059)
<i>Psychotria</i> spp.	Coleoptera	0.58 (±0.020)	
	Lepidoptera	0.57 (±0.059)	
	Orthopteroids	0.54 (±0.076)	
	Total	0.57 (±0.029)	
			0.45 (±0.006)
Plant genera	Coleoptera	–0.237	
	Lepidoptera	–0.328	0.09 (±0.005)
	Orthopteroids	0.018	0.53 (±0.007)
	Total	–0.165	0.37 (±0.004)

r, Spearman correlation between the phylogenetic distance of plants and the overlap of their herbivore communities measured by the Sorensen index. Significant values ($P < 0.05$, Mantel test) are in bold; data for *Psychotria* were too limited for calculation. So, average value of the Sorensen index for all pairwise comparisons between communities from different hosts.

of 50,734 herbivores comprising 935 species in a tropical lowland forest in New Guinea.

An analysis of herbivore specificity compared the degree of faunal overlap between communities feeding on closely and distantly related plants (Fig. 1). Any two host species from within the genera *Ficus* and *Psychotria* shared ~50% of their herbivorous species (Fig. 2, Table 1). This overlap between herbivore communities decreased gradually with increasing phylogenetic distance between hosts. The same was true for each of the three major herbivorous groups, including moths and butterflies (Lepidoptera), beetles (Coleoptera) and grasshoppers and stick insects (orthopteroids). Of 210 *Ficus* and *Psychotria* herbivores with known host range, only one species of *Coenobius* (Chrysomelidae) feeding on *Ficus nodosa* was monophagous. Large overlap among neotropical herbivore communities on congeneric hosts was found also in *Passiflora*⁹ (but see ref. 10).

We postulate that monophagous herbivores are rare in tropical forests because large genera tend to dominate tropical floras on all spatial scales, and our data demonstrate that monophagy is scarce in such genera. For example, Rubiaceae, the world's largest family of predominantly tropical woody plants, includes 15 genera with at least 100 species, representing 42% of 10,200 species in the family¹¹. These genera also represent 50% of Rubiaceae in Costa Rica¹², 39% in New Guinea¹³ and 36–58% in four neotropical forest communities¹⁴. Furthermore, large genera feature prominently in most tropical plant families¹¹.

The overlap between herbivore communities from phylogenetically distant hosts was also high, although lower than in congeneric hosts (Fig. 2, Table 1). Communities of Coleoptera and orthopteroids maintained high overlap (more than 40%) even between distantly related hosts. Most species of Coleoptera (96%) fed on the foliage as adults only. The key determinant of the distribution for these species is probably the host range of their poorly known wood-boring or root-feeding larvae¹⁵. The overlap between orthopteroid communities did not even depend on phylogenetic distance between hosts, as many species had broad host ranges spanning multiple plant families. In contrast, communities of Lepidoptera feeding on plants from different genera and families were highly distinct, with a low overlap (less than 20%) that decreased steeply towards zero with increasing phylogenetic distance between hosts. The narrow host specificity of larval Lepidoptera in New Guinea is consistent with data from the neotropics^{3,16}.

Large (speciose) plant genera hosted more distinct leaf-chewing communities than small genera, as indicated by a negative correlation (Spearman $r = -0.38$, $P < 0.05$, $n = 29$, Independent Contrasts) between the size of the plant genus in New Guinea¹³ and the average overlap (S_o) of its herbivore community with communities feeding on other genera. The overall relationship was due to

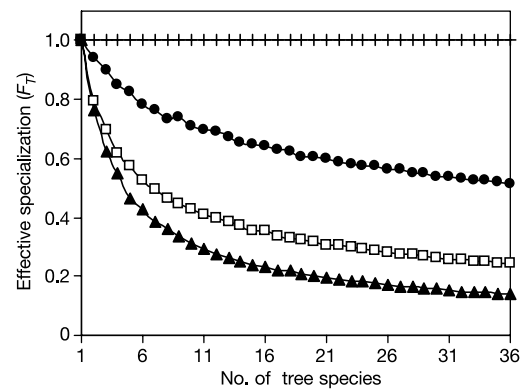


Figure 3 Dependence of effective specialization (F_T) on the number of host tree species studied. Samples from individual tree species, each from a different genus, were amalgamated in randomized sequence; the mean from 100 random sequences is given. Coleoptera (squares), Lepidoptera (circles), orthopteroids (triangles) and butterflies (crosses) are shown separately.

Coleoptera ($r = -0.45$, $P < 0.05$) and Lepidoptera ($r = -0.39$, $P < 0.05$); no correlation existed in the orthopteroids ($P > 0.5$). These findings contradict the hypothesis that taxonomically isolated plants from small genera support particularly distinct herbivore communities. We suggest that herbivores specialize more often on large plant genera than small ones because the former represent a large resource base, particularly because many herbivores have broad congeneric host ranges.

Our study showed that most herbivores feed on several closely related congeneric plant species. Many herbivores have even wider host ranges, as communities from allogeneric hosts shared on average 37% of all herbivorous species (Table 1). These results are at variance with the notion of extremely specialized interactions between herbivores and finely divided plant resources that provide distinct ecological niches and permit the coexistence of numerous herbivorous species in diverse tropical forests. Our findings suggest that alternative, non-equilibrium models of tropical diversity⁶ should be seriously considered.

There were on average $S_{av} = 32.9 \pm 1.7$ species of Coleoptera, 26.1 ± 2.4 species of Lepidoptera and 20.6 ± 0.9 species of orthopteroids feeding on a single host tree species. Their effective specialization¹⁷ was respectively $F_T = 0.24$ for Coleoptera, 0.51 for Lepidoptera and 0.14 for orthopteroids (Fig. 3) so that there were $S_{av}F_T = 7.9$ unique (effectively specialized) species of Coleoptera, 13.3 of Lepidoptera and 2.9 of orthopteroids per host tree species. These results are similar to the two to five unique species of

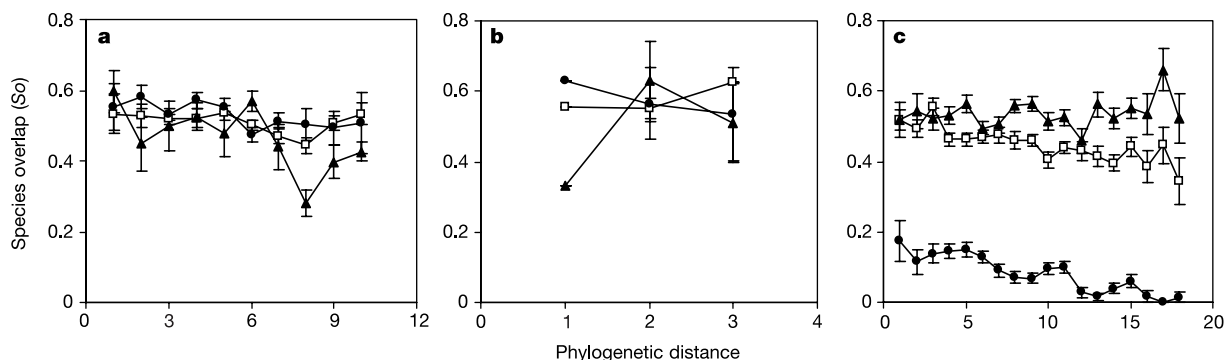


Figure 2 Overlap in species composition of their leaf-chewing communities and phylogenetic distance between *Ficus* spp. (a), *Psychotria* spp. (b) and plants from different genera (c). Values of Sorensen index S_o (means \pm s.e.m.) are shown for all pairwise

comparisons between hosts separated by a particular phylogenetic distance. Coleoptera (squares), Lepidoptera (circles) and orthopteroids (triangles) are shown separately.

Table 2 Estimates of the global number of arthropod species

Variable	1	Coleoptera 2	3	Lepidoptera
A. No. of herbivore species per tree species	682*	32.9†	32.9†	30.8¶
B. Effective specialization	0.20*	0.24†	0.24†	0.51†
C. Correction for non-herbivorous species	1.20*	1.20*	1.20*	1.00†
D. Proportion of species from arthropods	0.40*	0.23‡	0.23‡	0.10#
E. Proportion of canopy fauna from total	0.66*	0.42‡	0.42‡	1.00†
F. No. of tropical tree species	50,000*	50,000*		
G. No. of plant genera in New Guinea			1,872§	1,872§
H. Proportion of New Guinea species from total			0.05	0.05
Global no. of arthropod species ($A \times B \times C \times F / (D \times E)$)	31,000,000	4,904,348		
Global no. of arthropod spp. ($(A \times B \times C \times G) / (D \times E \times H)$)			3,672,376	5,881,075

Sources: *, from ref. 1; †, from this study; ‡, from ref. 4; §, from ref. 13; ||, from ref. 19; ¶, our estimate of 26.1 externally feeding leaf-chewer species multiplied by 1.18 to account for herbivorous species from other guilds, based on ref. 30; #, from ref. 8. Coleoptera 1, estimate from ref. 1. Note that the Coleoptera 2 and Lepidoptera estimates are mutually independent because they are based on two entirely different sets of parameters.

Coleoptera per host tree reported from other tropical forests^{4,18} but at variance with the 136 unique species postulated by Erwin¹. Our estimates of effective specialization are robust, as they changed little with increasing number of tree species included in the analysis (Fig. 3). In contrast, S_{av} values were underestimated, as the species accumulation curves for individual tree species did not approach an asymptote, indicating that the total species richness of their herbivore assemblages had not been sampled.

These host specificity data, together with improved estimates of some other parameters⁴, permitted a revision of Erwin's¹ estimate of arthropod diversity from 31.0 to 4.9 million species (Table 2). Erwin extrapolated the species richness of a herbivore community from a single tree species to the total number of tropical tree species. We refined Erwin's method in recognition of the fact that plant genera, rather than species, are more appropriate units for estimating the diversity of tropical herbivore communities. The average number of herbivorous species per host species was multiplied by the 1,872 plant genera known from New Guinea¹³ to obtain an estimate of arthropod species in New Guinea. The New Guinean diversity was extrapolated to global, using an estimate¹⁹ that ~5% of the world diversity in several well-known groups of organisms, including flowering plants, various invertebrates and vertebrates, occur in New Guinea. The total arthropod diversity was estimated at 3.7 million and 5.9 million species from data on Coleoptera and Lepidoptera, respectively (Table 2). This method was verified by using butterflies, a well-known taxon with 959 described species in New Guinea²⁰ and 15,000–20,000 worldwide⁵. In our samples, butterflies had $S_{av} = 0.63$ species per host tree species and effective specialization $F_T = 1.0$, leading to an estimate of 1,179 species in New Guinea and 23,500 worldwide.

Our estimates of global diversity are similar to the 6.6 million (ref. 18) and 4.8 million (ref. 4) species of arthropods extrapolated from other communities of tropical beetles. Even more importantly, they are also in agreement with estimates of ~5 million species based on the analysis of regional faunas and the evaluation of museum collections by taxonomists^{5,7,8,21}, reconciling the disparate results of ecological and taxonomic approaches to the estimation of global species diversity. □

Methods

Insects

Leaf-chewing insects feeding on 51 woody species (Fig. 1 and the following species of Euphorbiaceae s.l.: *Breynia cernua*, *Endospermum labios*, *Homalanthus novoguineensis*, *Mallotus mollissimus*, *Melanolepis multiglandulosa* and *Pimelodendron amboinicum*) from all major lineages of flowering plants and all stages of rainforest secondary succession²² were studied at four study sites in a diverse lowland rainforest (>150 woody species per hectare) near Madang in Papua New Guinea in 1994–2000 (ref. 23). Insects were collected by hand from foliage of each plant species for at least 1 year. Collecting effort was 1,500 m² of foliage sampled and >1,000 tree inspections (a particular tree sampled at a particular time) per species. This sampling effort represented ~1,000 person-days of fieldwork. Each insect was provided with leaves of the plant species from which it was collected and only those that fed were retained in the analyses. Larvae were reared to adults whenever possible. All insects were assigned to morphospecies and later verified and identified by

specialists as far as possible (http://www.nmnh.si.edu/new_guinea/). Vouchers are deposited in the Bishop Museum, Honolulu, and the Smithsonian Institution, Washington.

Feeding

All feeding records limited to a single individual on a particular host species were excluded as poorly supported. Monophagous species were defined as those represented by at least 10 individuals, all feeding on a single plant species. Faunal overlap was examined in 45 host taxa with known phylogenetic relationships. Overlap was measured by the Sørensen coefficient $So = 2a / (2a + b + c)$, where a is the number of species common to the two compared samples, and b and c are the numbers of species present only in the first and second samples respectively. So quantifies the proportion of species in a sample that also occurs in the other sample, averaged over the two samples.

The average proportion of herbivorous species feeding on a particular host plant that was unique (effectively specialized¹⁷) to that plant was estimated as $F_T = S_{\eta} / H_T$, that is, as the ratio of the total number of herbivorous species found on all T hosts studied (S_{η}), divided by the number of host-plant records involving these hosts (H_T). The effective specialization and species richness of herbivores were estimated from communities feeding on 36 tree species, each representing a different genus.

Phylogenetic relationships

Phylogenetic distance between plants was estimated as the number of nodes in the phylogeny between these plant species. Separate correlation analyses of 13 *Ficus* spp., 4 *Psychotria* spp. and 30 plants from different genera were performed. *Ficus wassa* and *Psychotria micralabastra* represented their respective genera in the latter analysis. COMPARE 4.4 software was used to calculate independent contrasts.

Phylogenetic relationships of plant species were obtained from refs 24–28. All Rubiaceae species were sequenced for large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase gene (*rbcL*) and 30S ribosomal protein S16 gene (*rps16*). The data were analysed under parsimony with PAUP, version 4.0d64* (D. Swofford, unpublished). All relationships were highly supported and in agreement with earlier analyses of the family²⁹. Accession numbers from the EMBL/GenBank data bases for the *rps16* intron and the *rbcL* gene, respectively, follow (see Fig. 1 for full plant names): *G. hansemanii*, AJ320077, AJ318446; *M. citrifolia* chloroplast, AJ320078, AJ318448; *M. scratchleyi*, AJ320079, AJ318447; *N. orientalis*, AJ320080, AJ318449; *N. clemensiae*, AJ320081, AJ318450; *P. platyclada*, AJ320082, AJ318451; *P. leptothyrsa*, AJ320083, AJ318452; *P. micralabastra*, AJ320084, AJ318453; *P. micrococca*, AJ320085, AJ318454; *P. ramuensis*, AJ320086, AJ318455; *R. schumanniana*, AJ320087, AJ318456; *T. buruensis*, AJ320088, AJ318457; *T. timon*, AJ320089, AJ318458; *V. cauliflora*, AJ320090, AJ318459.

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- Erwin, T. L. Tropical forests: their richness in Coleoptera and other species. *Coleopterist's Bull.* **36**, 74–75 (1982).
- Basset, Y. Host specificity of arboreal and free-living insect herbivores in rain forests. *Biol. J. Linn. Soc.* **47**, 115–133 (1992).
- Barone, J. A. Host-specificity of folivorous insects in a moist tropical forest. *J. Anim. Ecol.* **67**, 400–409 (1998).
- Ødegaard, F. How many species of arthropods? Erwin's estimate revised. *Biol. J. Linn. Soc.* **71**, 583–597 (2000).
- Stork, N. E. How many species are there? *Biodivers. Conserv.* **2**, 215–232 (1993).
- Hubbell, S. P. *The Unified Neutral Theory of Biodiversity and Biogeography* (Princeton Univ. Press, Princeton, 2001).
- May, R. M. in *Nature and Human Society: The Quest for a Sustainable World* (eds Raven, P. H. & Williams, T.) 30–45 (National Academy Press, Washington DC, 2000).
- Nielsen, E. S. & Mound, L. A. in *Nature and Human Society: The Quest for a Sustainable World* (eds Raven, P. H. & Williams, T.) 312–322 (National Academy Press, Washington DC, 2000).
- Thomas, C. D. Herbivore diets, herbivore colonization, and the escape hypothesis. *Ecology* **71**, 610–615 (1990).
- Marquis, R. J. in *Plant–Animal Interactions: Evolutionary Ecology in Tropical and Temperate Regions* (eds Price, P. W., Lewinsohn, T. M., Fernandes, G. W. & Benson, W. W.) 179–208 (Wiley, London, 1991).
- Mabberley, D. J. *The Plant Book* (Cambridge Univ. Press, Cambridge, 1987).
- Burger, W. & Taylor, C. M. Flora Costaricensis: Family #202 Rubiaceae. *Fieldiana Botany*, N.S. **33**, 1–333 (1993).

13. Höft, R. *Plants of New Guinea and the Solomon Islands. Dictionary of the Genera and Families of Flowering Plants and Ferns* (Wau Ecology Institute, Wau, 1992).
14. Gentry, A. H. (ed.) *Four Neotropical Forests* (Yale Univ. Press, New Haven, 1990).
15. Jolivet, P. & Hawkeswood, T. J. *Host-Plants of Chrysomelidae of the World* (Backhuys, Leiden, 1995).
16. Janzen, D. H. Ecological characterization of a Costa Rican dry forest caterpillar fauna. *Biotropica* **20**, 120–135 (1988).
17. May, R. M. How many species? *Phil. Trans. R. Soc. Lond. B* **330**, 293–304 (1990).
18. Basset, Y., Samuelson, G. A., Allison, A. & Miller, S. E. How many species of host-specific insects feed on a species of tropical tree? *Biol. J. Linn. Soc.* **59**, 201–216 (1996).
19. Sekhran, N. & Miller, S. E. (eds) *Papua New Guinea Country Study on Biological Diversity* (Papua New Guinea Dept of Environment & Conservation, Waigani, Papua New Guinea, 1996).
20. Parsons, M. *The Butterflies of Papua New Guinea* (Academic, London, 1999).
21. Miller, S. E., Novotny, V. & Basset, Y. in *Foundations of Tropical Biology: Key Papers and Commentaries* (eds Chazdon, R. L. & Whitmore, T. C.) (Univ. Chicago Press, Chicago, in the press).
22. Leps, J., Novotny, V. & Basset, Y. Habitat and successional optimum of plants and the composition of their leaf-chewing herbivores in Papua New Guinea. *J. Ecol.* **89**, 186–199 (2001).
23. Basset, Y., Novotny, V., Miller, S. E. & Pyle, R. Quantifying biodiversity: experience with parataxonomists and digital photography in Papua New Guinea and Guyana. *BioScience* **50**, 899–908 (2000).
24. Angiosperm Phylogeny Group An ordinal classification for the families of flowering plants. *Ann. Missouri Bot. Gard.* **85**, 531–553 (1998).
25. Weiblen, G. D. Phylogenetic relationships of functionally dioecious *Ficus* (Moraceae) based on ribosomal DNA sequences and morphology. *Am. J. Bot.* **87**, 1342–1357 (2000).
26. Backlund, M., Oxelman, B. & Bremer, B. Phylogenetic relationships within the Gentianales based on ndhF and rbcL sequences, with particular reference to the Loganiaceae. *Am. J. Bot.* **87**, 1029–1043 (2000).
27. Soltis, D. E. *et al.* Angiosperm phylogeny inferred from 18S rDNA, rbcL, and atpB sequences. *Bot. J. Linn. Soc.* **133**, 381–461 (2000).
28. Qiu, Y.-L. *et al.* The earliest angiosperms. Evidence from mitochondrial, plastid and nuclear genomes. *Nature* **402**, 404–407 (1999).
29. Bremer, B. *et al.* More characters or more taxa for a robust phylogeny—case study from the coffee family (Rubiaceae). *Syst. Biol.* **48**, 413–435 (1999).
30. Powell, J. A., Mitter, C. & Farrell, B. in *Lepidoptera, Moths and Butterflies Vol. 1: Evolution, Systematics, and Biogeography* (ed. Kristensen, N. P.) 403–422 (Walter de Gruyter, Berlin, 1998).

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Correspondence and requests for materials should be addressed to V.N.
(e-mail: novotny@entu.cas.cz).

Developmental constraints versus flexibility in morphological evolution

Patricia Beldade, Kees Koops & Paul M. Brakefield

Institute of Evolutionary and Ecological Sciences, Leiden University,
PO Box 9516, 2300 RA Leiden, The Netherlands

Evolutionary developmental biology has encouraged a change of research emphasis from the sorting of phenotypic variation by natural selection to the production of that variation through development¹. Some morphologies are more readily generated than others, and developmental mechanisms can limit or channel evolutionary change². Such biases determine how readily populations are able to respond to selection³, and have been postulated to explain stasis in morphological evolution⁴ and unexplored morphologies⁵. There has been much discussion about evolutionary constraints^{6–8} but empirical data testing them directly are

sparse^{9,10}. The spectacular diversity in butterfly wing patterns¹¹ is suggestive of how little constrained morphological evolution can be. However, for wing patterns involving serial repeats of the same element, developmental properties suggest that some directions of evolutionary change might be restricted^{12,13}. Here we show that despite the developmental coupling between different eyespots in the butterfly *Bicyclus anynana*, there is great potential for independent changes. This flexibility is consistent with the diversity of wing patterns across species and argues for a dominant role of natural selection, rather than internal constraints, in shaping existing variation.

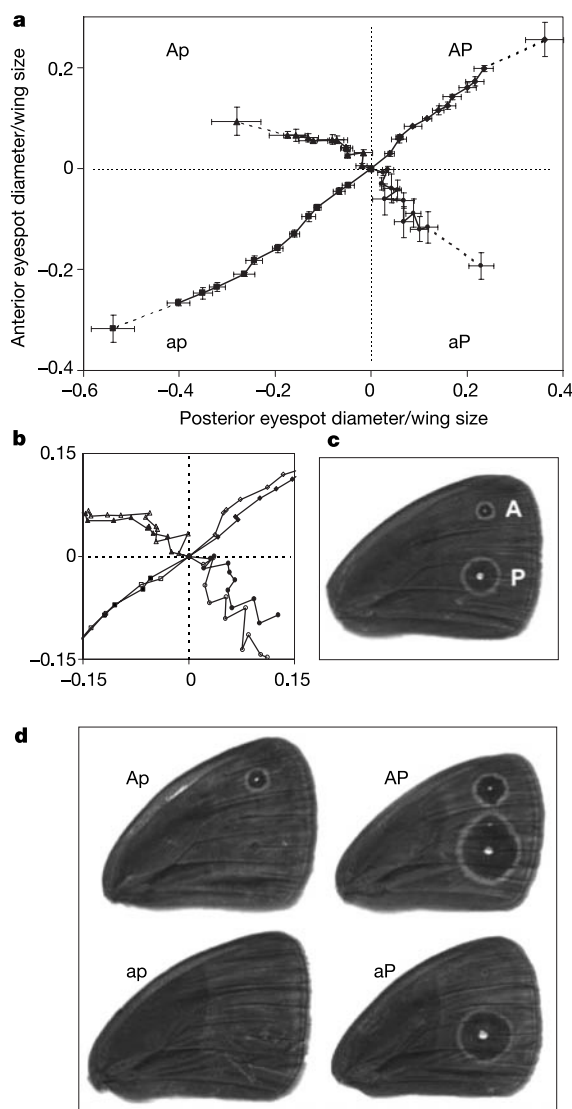


Figure 1 Response to artificial selection on the size of the dorsal forewing eyespots of *B. anynana*. **a**, Eyespot diameter/wing size relative to unselected control values are given for the different directions of selection. AP and ap are coupled directions; Ap and aP are uncoupling directions. Each point represents the mean (\pm standard error) for the two replicate lines for each generation. Solid lines join points covering the first 11 consecutive generations, all starting from the same original population (centre of graphic, G0). Broken lines join the points for G11 and G25 phenotypes. **b**, Enlargement of the central area of **a** showing the behaviour of individual replicate lines (filled and open symbols). **c**, Typical dorsal surface of forewing of unselected female showing the anterior (A) and posterior (P) eyespots. **d**, Representative G25 phenotypes (most ap females have no eyespots and many AP females have extra, satellite eyespots). Responses in males were comparable to those in females.