

of global climate models^{9,10}. Thus, there might be a greater frequency of transition between ANPP limitation by water and by other limiting resources. A key result would be reductions in ANPP that were greater than expected, as well as greater variability, than that predicted by site-level models alone—even in biomes previously considered insensitive (for example, forests)—in response to future climate. □

Methods

We searched for data from a variety of sources, but included only those data sets with at least six years of concurrent measures of annual precipitation and ANPP. We assembled data from 14 sites that met these criteria, including ten US Long-Term Ecological Research (LTER) Network sites⁷, and sites in Rock Valley (RCR; desert), Nevada²⁹, Jasper Ridge Biological Preserve (JR; Mediterranean grassland), California¹⁴, Patagonia Steppe (PSA; grass/shrub steppe), Argentina³⁰, and Barro Colorado Island (BCI; tropical forest), Republic of Panama. These sites represent a broad gradient of precipitation in North and South America (105–2,542 mm MAP). The LTER sites are listed in ref. 7, and include Bonanza Creek, Alaska (BNZ), Cedar Creek, Minnesota (CDR), Harvard Forest, Massachusetts (HFR), Hubbard Brook, New Hampshire (HBF), Jornada, New Mexico (JRN), Kellogg, Michigan (KBS), Konza Prairie, Kansas (KNZ), Sevilleta, New Mexico (SEV), and Shortgrass Steppe, Colorado (SGS). We added the H.J. Andrews Experimental Forest, Oregon (AND), to this LTER data set. Data for BCI were obtained from the Oak Ridge National Laboratory Distributed Active Archive Center (ORNL DAAC at http://www.eodis.ornl.gov/npp/npp_home.html).

Received 13 January; accepted 15 April 2004; doi:10.1038/nature02561.

- Rosenzweig, M. L. Net primary productivity of terrestrial communities: prediction from climatological data. *Am. Nat.* **102**, 67–74 (1968).
- Lieth, H. in *Primary Productivity of the Biosphere* (eds Lieth, H. & Whittaker, R. H.) 237–263 (Springer, New York, 1975).
- Webb, W. L., Lauenroth, W. K., Szarek, S. R. & Kinerson, R. S. Primary production and abiotic controls in forests, grasslands, and desert ecosystems of the United States. *Ecology* **64**, 134–151 (1986).
- Sala, O. E., Parton, W. J., Joyce, L. A. & Lauenroth, W. K. Primary production of the central grassland region of the United States. *Ecology* **69**, 40–45 (1988).
- Walter, H. Grassland, Savanne und Busch der arideren Teile Afrikas in ihrer ökologischen Bedingtheit. *Jb. Wiss. Bot.* **87**, 750–760 (1939).
- Paruelo, J. M., Lauenroth, W. K., Burke, I. C. & Sala, O. E. Grassland precipitation–use efficiency varies across a resource gradient. *Ecosystems* **2**, 64–68 (1999).
- Knapp, A. K. & Smith, M. D. Variation among biomass in temporal dynamics of aboveground primary production. *Science* **291**, 481–484 (2001).
- Veron, S. V., Paruelo, J. M., Sala, O. E. & Lauenroth, W. K. Environmental controls of primary production in agricultural systems of the Argentine Pampas. *Ecosystems* **5**, 625–635 (2002).
- IPCC. *Climate Change 2001: Synthesis Report. A Contribution of Working Groups I, II, and III to the Third Assessment Report of the Intergovernmental Panel on Climate Change* (eds Watson, R. T. and Core Writing Team) (Cambridge Univ. Press, Cambridge, 2001).
- Easterling, D. R. *et al.* Climate extremes: Observations, modeling, and impacts. *Science* **289**, 2068–2074 (2000).
- Schimel, D. S. *et al.* Recent patterns and mechanisms of carbon exchange by terrestrial ecosystems. *Nature* **414**, 169–172 (2001).
- Noy-Meir, I. Desert ecosystems: environment and producers. *Annu. Rev. Ecol. Syst.* **4**, 51–58 (1973).
- Smith, S. D. *et al.* Elevated CO₂ increases productivity and invasive species success in an arid ecosystem. *Nature* **408**, 79–82 (2000).
- Shaw, M. R. *et al.* Grassland responses to global environmental changes suppressed by elevated CO₂. *Science* **298**, 1987–1990 (2002).
- Lauenroth, W. K. & Sala, O. E. Long-term forage production of North American shortgrass steppe. *Ecol. Appl.* **2**, 397–403 (1992).
- Grime, J. P. Evidence for existence of 3 primary strategies in plants and its relevance to ecological and evolutionary theory. *Am. Nat.* **111**, 1169–1194 (1977).
- Ehleringer, J. R. in *Terrestrial Global Productivity* (eds Roy, J., Saugier, B. & Mooney, H. A.) 345–362 (Academic, San Diego, 2001).
- Seastedt, T. R. & Knapp, A. K. Consequences of nonequilibrium resource availability across multiple time scales: the transient maxima hypothesis. *Am. Nat.* **141**, 421–433 (1993).
- Austin, A. T. & Vitousek, P. M. Nutrient dynamics on a precipitation gradient in Hawai'i. *Oecologia* **113**, 519–529 (1998).
- Chapin, F. S. III, Matson, P. A. & Mooney, H. A. *Principles of Terrestrial Ecosystem Ecology* (Springer, New York, 2002).
- Knapp, A. K. *et al.* Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science* **298**, 2202–2205 (2003).
- Schlesinger, W. H. *Biogeochemistry: An Analysis of Global Change* (Academic, San Diego, 1997).
- Smith, S. D., Monson, R. K. & Anderson, J. E. *Physiological Ecology of North American Desert Plants* (Springer, New York, 1997).
- Aber, J. *et al.* Nitrogen saturation in temperate forest ecosystems. *Bioscience* **48**, 921–934 (1998).
- Ehleringer, J. R. & Mooney, H. A. in *Encyclopedia of Plant Physiology (New Series)* vol 12C (eds Lange, O. L., Nobel, P. S., Osmond, C. B. & Ziegler, H.) 205–231 (Springer, New York, 1983).
- Schlesinger, W. H. *et al.* Biological feedbacks in global desertification. *Science* **247**, 1043–1048 (1990).
- Reynolds, J. F., Virginia, R. A., Kemp, P. R., de Soyza, A. G. & Tremmel, D. C. Impact of drought on desert shrubs: effects of seasonality and degree of resource island development. *Ecol. Monogr.* **69**, 69–106 (1999).
- Le Houerou, H. N., Bingham, R. L. & Skerbek, W. Relationship between the variability of primary production and the variability of annual precipitation in world arid lands. *J. Arid Environ.* **15**, 1–18 (1998).

- Turner, F. B. & Randall, D. C. Net production by shrubs and winter annuals in southern Nevada. *J. Arid Environ.* **17**, 23–36 (1989).
- Jobbagy, E. G. & Sala, O. E. Controls of grass and shrub aboveground production in the Patagonian steppe. *Ecol. Appl.* **10**, 541–549 (2000).

Supplementary Information accompanies this paper on www.nature.com/nature.

Acknowledgements We thank J. Bronstein, J. Cable, G. Davidowitz, A. Eilts, B. Enquist, D. Ignace, A. Kerckhoff, D. Potts, D. Schimel, L. Venable and M. Pavao-Zuckerman for comments on the manuscript. This work derived from an NCEAS working group, PrecipNet (principal investigator M.E.L.), supported by the National Science Foundation, the University of California, and the Santa Barbara campus. We acknowledge the support of the United States Department of Energy, the National Park Service, the National Science Foundation, and the United States Department of Agriculture.

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

Correspondence and requests for materials should be addressed to T.E.H. (huxman@email.arizona.edu) or M.D.S. (melinda.smith@yale.edu).

Harmonic-hopping in Wallacea's bats

Tigga Kingston^{1*} & Stephen J. Rossiter^{2,3*}

¹Department of Geography, Boston University, Massachusetts 02215, USA

²School of Biological Sciences, Queen Mary, University of London, London E1 4NS, UK

³School of Biological Sciences, University of Bristol, Bristol BS8 1UG, UK

* These authors contributed equally to this work

Evolutionary divergence between species is facilitated by ecological shifts, and divergence is particularly rapid when such shifts also promote assortative mating^{1–3}. Horseshoe bats are a diverse Old World family (Rhinolophidae) that have undergone a rapid radiation in the past 5 million years⁴. These insectivorous bats use a predominantly pure-tone echolocation call matched to an auditory fovea (an over-representation of the pure-tone frequency in the cochlea and inferior colliculus^{5,6}) to detect the minute changes in echo amplitude and frequency generated when an insect flutters its wings⁷. The emitted signal is the accentuated second harmonic of a series in which the fundamental and remaining harmonics are filtered out⁸. Here we show that three distinct, sympatric size morphs of the large-eared horseshoe bat (*Rhinolophus philippinensis*) echolocate at different harmonics of the same fundamental frequency. These morphs have undergone recent genetic divergence, and this process has occurred in parallel more than once⁹. We suggest that switching harmonics creates a discontinuity in the bats' perception of available prey that can initiate disruptive selection¹. Moreover, because call frequency in horseshoe bats has a dual function in resource acquisition and communication, ecological selection on frequency might lead to assortative mating and ultimately reproductive isolation and speciation, regardless of external barriers to gene flow^{1–3}.

The large-eared horseshoe bat (*Rhinolophus philippinensis*) is a rare species found from the Wallacea region of southeast Asia to northeast Australia. Observed variation in body size across its range has led to suggestions that more than one species might be present, currently recognized as subspecies¹⁰. A previous study of two size forms from Queensland, calling at 40 and 28 kHz, revealed a polyphyletic origin¹¹. We used acoustic and genetic analyses to determine the basis of phenotypic variation in this species. Bats were captured on Buton Island, southeast Sulawesi, with an additional individual from neighbouring Kabaena Island. Intensive trapping over four summers revealed low numbers of three discrete size morphs ($n = 24$) (Fig. 1).

Each morph was characterized by external features diagnostic for the species, but adults differed substantially in both forearm length (mean \pm s.d. (n) for the large morph, 56.1 ± 1.5 mm (7); small morph, 47.0 ± 0.4 mm (13); intermediate (Buton island), 50.6 ± 1.4 mm (3); intermediate (Kabaena island), 48.4 mm) and body mass (large, 11.1 ± 0.5 g (7); small, 6.7 ± 0.4 g (13); Buton intermediate, 8.4 ± 0.4 g (3); Kabaena intermediate, 7.0 g). The largest morph was thus almost twice as heavy as the smallest, with a forearm difference of nearly 10 mm. No sexual dimorphism in size was observed within morphs.

Each size morph was associated with a different echolocation call frequency (large, 27.2 ± 0.2 kHz (6); small, 53.6 ± 0.6 kHz (11); Buton intermediate, 39.0 ± 0.8 kHz (3); Kabaena intermediate, 41.7 kHz). Remarkably, the emitted frequencies of the intermediate and small morphs correspond to the third and fourth harmonics of the large morph's fundamental frequency respectively, and the fundamental of the small morph matches the second harmonic of the large morph's fundamental frequency (Fig. 2). The frequency differences in these bats therefore seem to result from some form of 'harmonic-hopping'.

Switching harmonics is likely to have far-reaching consequences for the ecological interactions between morphs by creating a marked discontinuity in the bats' perception of available prey. Low frequencies have longer wavelengths, so they reflect poorly from small prey because of Rayleigh scattering^{12–14}. Consequently, the large morph is likely to encounter difficulties in detecting insects with wing lengths of less than 12.8 mm (wavelength 27.2 kHz under local conditions), and experimental evidence suggests that insects smaller than 5.0 mm might be undetectable to bats echolocating at 27 kHz^{13,14}. In contrast, prey almost half the size should still be detectable by the small morph (wavelength 6.5 mm) and possibly by the intermediate morphs (8.9 mm). However, low frequencies are less subject to environmental attenuation¹⁵, conferring greater detection ranges. We modelled prey detection distances for the three morphs from Buton for small (5 mm) and large (25 mm) prey. Backscattering reduces the target strength of small prey so severely at low frequencies that the detection distances differ little between morphs and are uniformly low (1.6–2.5 m). By comparison, atmospheric attenuation is the key factor influencing detection ranges of the large prey, with detection distances of 9.6 m for the large morph, 7.3 m for the intermediate morph and 5.5 m for the small morph. The large morph therefore samples more than five times the volume of potential 'large-prey space' than the small morph, over twice that of the intermediate, and, most importantly, more than 90 times the

volume of its own 'small prey space'. Switching harmonics could therefore create a marked shift in 'apparent resource distribution'; to the large morph, large insects appear more numerous than they are in absolute terms, and small prey (if detected) appear less abundant. Conversely, the small morph will detect almost nine times as many small insects as the large morph. This perceptual ecological discontinuity provides a novel opportunity for natural selection to initiate divergence within the population²; 54 kHz bats detect more small prey, but 27 kHz bats are at a competitive advantage in taking large insects.

Divergent ecological selection can, in turn, lead to reproductive isolation if the trait under selection also influences mate recognition and results in assortative mating^{1–3}. Selection for a clear communication channel has been implicated in acoustic divergence in cryptic bat species¹⁶, and in horseshoe bats, intraspecific communication seems to involve the temporal and phasic arrangement of the constant-frequency (CF) component of the call^{17,18}, with a repertoire of oral emissions below the fundamental¹⁹. Harmonic switching will not only shift the constant-frequency signal of each morph out of the sensitive foveal region of the other, but into areas of response minima. An acoustic 'blind spot' (auditory threshold maximum) at the fundamental frequency^{18,20} will greatly reduce the sensitivity of the small morph to the large morph's 27 kHz calls. Similarly, the large morph will be unresponsive to constant-frequency calls at 54 kHz because of a rapid decrease in sensitivity to frequencies above the auditory fovea²⁰. The large and small morphs are thus functionally deaf to each other's constant-frequency calls. Although the intermediate morph should be able to hear frequencies used by the large morph, and be heard by the small morph, in neither case is this receptivity reciprocal. Similarly, the response minima at the fundamental frequency are likely to disrupt the perception of low-frequency vocalizations among morphs. Thus, if we assume that acoustic communication is important for mate recognition, as occurs typically in bats²¹, harmonic-hopping could provide an intrinsic mechanism of instantaneous pre-mating isolation.

To test for assortative mating between morphs, we examined variation at microsatellite loci (see Supplementary Table 1) and found significant genetic differentiation between the large and small morphs ($F_{st} = 0.18$, $P < 0.001$) at a level commonly detected among island bat populations, providing strong evidence against panmixia. Despite this, the large and small morphs showed greater genetic similarity to each other than to other congeners caught at the same site (small and large morphs versus Sulawesi horseshoe bat (*R. celebensis*), $F_{st} = 0.46$, $P < 0.001$ and $F_{st} = 0.41$, $P < 0.01$, respectively; small and large morphs versus broad-eared horseshoe bat (*R. euryotis*), $F_{st} = 0.43$, $P < 0.001$ and $F_{st} = 0.40$, $P < 0.001$, respectively). The intermediate was differentiated from both the large ($F_{st} = 0.09$, $P < 0.05$) and small ($F_{st} = 0.19$, $P < 0.001$) forms, even after the Kabaena individual was excluded. Although the small samples preclude a definitive test, these data indicate that reproductive isolation between morphs has evolved recently, or might still be evolving. Assortative mating between the morphs is also reflected in differences in maximum-likelihood estimates of heterozygosity (large, 0.84; small, 0.54; intermediate, 0.74) and, in particular, in the small overlap between the range of possible values (given by the likelihood curves) of the small and large morphs (95% confidence intervals: large morph, 0.56–0.96; small, 0.25–0.77; intermediate, 0.40–0.93). Moreover, the direction of this trend suggests that the large morph is the ancestral form, because reproductive isolation by harmonic hops would create a founder effect and a consequent loss of genetic diversity in the new phonic types.

We also compared mitochondrial DNA control-region haplotypes of the three morphs from Buton with published sequences from large and intermediate morphs from Queensland, Australia¹¹. A consensus tree based on parsimony analysis revealed a reciprocally

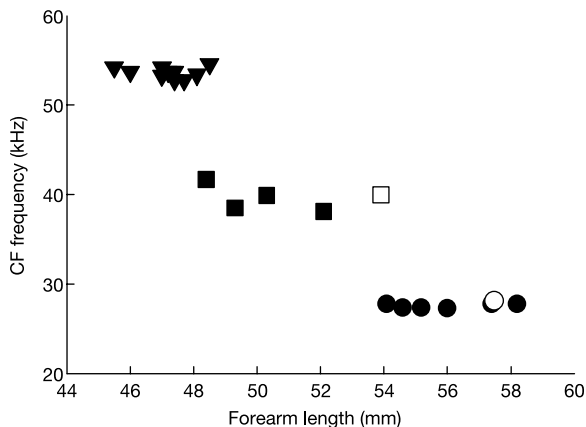


Figure 1 Relationship between constant-frequency component of calls and forearm length for three sympatric morphs of the large-eared horseshoe bat. Circle, large morph; triangle, small morph; square, intermediate morph. Filled symbols are from Sulawesi and open symbols are based on average forearm lengths³⁰ and call frequencies¹¹ from the two morphs present in Australia.

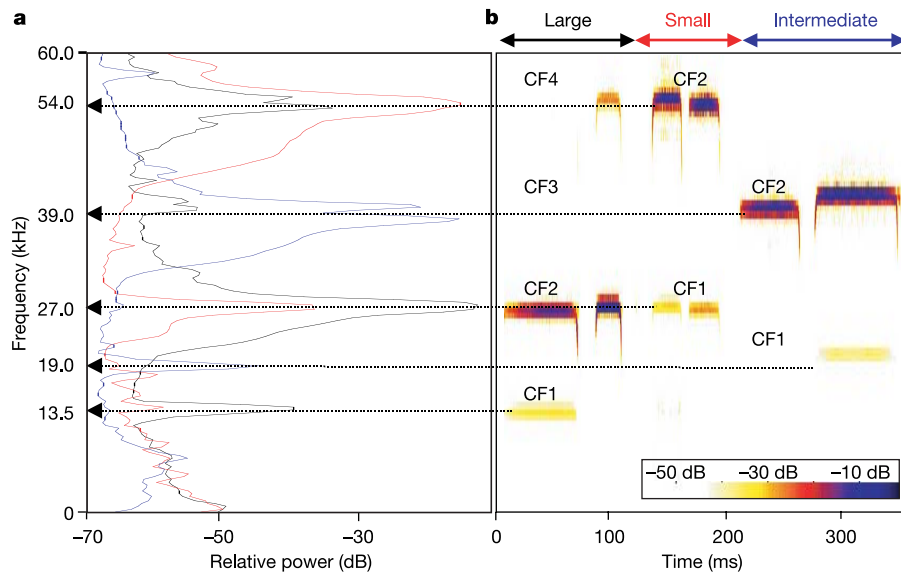


Figure 2 Echolocation calls of the three sympatric morphs of the large-eared horseshoe bat. **a**, Mean power spectra. Each includes three to eight calls per individual from 6 large morphs, 11 small morphs, and the 3 intermediate morphs from Buton. Black line, large morph; red line, small morph; blue line, Buton intermediate morph. **b**, Sonograms of two

calls from different individuals of each morph. The scale bar indicates relative power (dB). The constant-frequency harmonics of each morph are denoted by the prefix CF. CF1 is the fundamental and CF2 is the main frequency emitted. Dotted arrows indicate peak frequencies and corresponding harmonics.

monophyletic origin of both the Sulawesi and Australian morphs, with strong bootstrap support (Fig. 3). Pairwise sequence divergence (see Supplementary Table 2) was considerably lower among bats within clades (Queensland mean 0.78%, range 0.2–1.5%; Buton mean 1.06%, range 0–2.2%) than between clades (mean 7.93%, range 6.9–9.1%), indicating that distinct morphs of the large-eared horseshoe bat might have evolved more than once.

We propose that harmonic shifts provide new ecological opportunities and can promote social and thus genetic divergence regardless of the external barriers to gene flow. Remarkably, all the species of the *R. philippinensis* clade⁴, for which echolocation data are available, call at harmonics of the large morph's fundamental. *R. megaphyllus* is paraphyletic within *R. philippinensis* in Australia and echolocates at about 66 kHz¹¹ (large morph's fifth harmonic),

and *R. celebensis*, *R. virgo* and *R. borneensis* (from Sulawesi, the Philippines and Borneo, respectively) all call at about 81 kHz (sixth harmonic; ref. 22, and J. Sedlock, personal communication). We therefore suggest that harmonic shifts might have contributed to the rapid radiation of rhinolophid bats in southeast Asia, where about 30 species have originated within about 5 million years⁴. Although the origin of harmonic-hopping is unresolved, the fine tuning of the constant-frequency call to the auditory fovea necessitates a close association between the vocalization and auditory systems. We suspect that a change in cochlear dimensions initiates the switch because the functional onset and tuning of the cochlear filter are innate processes arising from postnatal maturation of the cochlea²³, which the vocalization system tracks by auditory feedback^{23,24}. Small shifts in cochlear width associated with constant-frequency shifts in horseshoe bats can occur independently of body size²². Thus, selection on cochlear width to alter the call frequency could initiate divergence and could be followed by adjustments in body size to exploit suitable prey. Such a system illustrates how, theoretically, micro-mutations could produce large-scale changes, and highlights the important function that sensory ecology is likely to have in speciation events. □

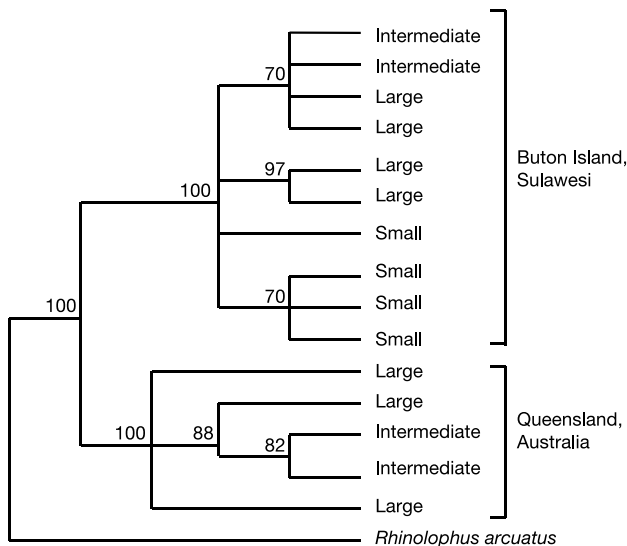


Figure 3 Consensus tree based on parsimony analysis of mtDNA haplotypes, showing phylogenetic relationships between large-eared horseshoe bat morphs sampled on Buton Island, Sulawesi, and Queensland, Australia. Bootstrap values (percentages from 1,000 replicates) are given on the nodes.

Methods

Bat capture and measurement

We undertook fieldwork on Buton Island and Kabaena Island, Sulawesi, Indonesia, from June to September over four consecutive years (2000–2003). Bats were captured in 7–14 harp traps positioned daily along forest trails within a 1-km² study plot, with the exception of one individual, which was caught with a hand net in a cave. On capture, each individual's sex, weight and forearm length were measured.

Analysis of echolocation calls

We recorded echolocation calls with a Pettersson D980 detector sampling at 400 kHz and a Pettersson D960 detector sampling at 350 kHz. Animals were held about 30 cm from the microphone for recording. Time-expanded (×10) outputs were recorded on a Sony WM-D6C Walkman cassette recorder and analysed with BatSound Pro Version 5. Power spectra were used to derive the frequency of maximum energy (kHz) and the associated harmonics for each call of each individual.

Calculation of prey size and detection distances

Mean wavelengths were calculated as the speed of sound in air divided by the call frequency, where the speed of sound is 347.65 m s⁻¹, assuming 25 °C and 80% relative humidity. Maximum target ranges were calculated as the distance at which sound losses totalled 120 dB (relative power of the emitted signal), assuming a detection threshold for *Rhinolophus* bats of 0 dB (ref. 20). The total sound loss includes absorption by the

atmosphere (attenuation) and spherical spreading losses, assuming that the bat acts as a point source. The rate of atmospheric absorption is dependent on temperature, humidity and frequency and was calculated as 0.57 dB m⁻¹ at 27.1 kHz, 1.09 dB m⁻¹ at 39.0 kHz, 1.23 dB m⁻¹ at 41.7 kHz, and 1.86 dB m⁻¹ at 53.6 kHz. Spreading losses are described by the standard inverse square law for point sources. If the target is a small insect that acts as a point source it will re-radiate the incident sound back to the bat, so spreading losses are doubled¹⁵.

Total sound losses for a target at $R_2 = 2(20 \log(R_1/R_2)) + a(2(R_2 - R_1))$ (in which the two terms are spherical spreading and absorption by atmosphere, respectively) if R_1 and R_2 are two distances at a point from the original source; we follow ref. 15 in assuming that R_1 is 0.1 m in front of the bat, R_2 is the target range (distance to target) and a is absorption loss per metre. The echo strength was then adjusted for target losses, which depend on the call frequency and size of the prey. We modelled detection distance for prey with wing lengths of 25 and 5 mm with frequency-dependent target losses incorporated^{13,14}.

Tissue collection for genetic analysis

Wing membrane samples were collected from all individuals with a 3-mm biopsy punch (Stiefel Laboratories), and DNA was isolated with Qiagen DNeasy kits.

Microsatellite analysis

We genotyped *R. philippinensis* morphs (6 large, 4 intermediate, 13 small), *R. euryotis* (7 from Buton, 6 from Kabaena) and *R. celebensis* (6 from Buton) with 9–12 polymorphic microsatellite markers developed from *R. ferrumequinum* (GenBank accession numbers AF160200, AF160202, AF160207, AF160210, AF160211 (ref. 25), AJ560694, AJ560695b, AJ560698, AJ560702–AJ560704 and AJ560710 (ref. 26)). Primers were fluorescently labelled and amplified products were run on an ABI 3700 automated sequencer. Allele sizes were analysed with the software GENESCAN version 3.1 and GENOTYPYPER version 3.6.

Allelic diversity (number of polymorphic loci, mean number of alleles per locus) recorded for the five taxa was as follows: large morph, 8, 3.08; small morph, 8, 2.67; intermediate morph, 8, 2.25; *R. euryotis* (Buton), 9, 6.1; *R. celebensis*, 10, 4.75. We calculated Weir–Cockerham *F* statistics and assessed their significance with the permutation routine in the program GENETIX, which is suited to the analysis of small sample sizes. *F*_{is} estimates derived for each locus and for all loci together showed no significant deviation (*P* > 0.05) from Hardy–Weinberg expectations in any of the five taxa. To determine whether heterozygosity estimates differed significantly between the three *R. philippinensis* morphs, we obtained likelihood curves by following the method described in detail in ref. 27, in which the precision of the maximum-likelihood estimate of heterozygosity is reflected in the spread of the curve. To check that possible sampling of close relatives did not bias our *F*_{st} estimates, we examined mtDNA haplotypes and derived Queller–Goodnight pairwise coefficients of relatedness between individuals within each size class by using the program RELATEDNESS version 5.0. In only two cases of shared mtDNA haplotypes, relatedness estimates approximated to zero.

Phylogenetic inference

We amplified and sequenced 460 base pairs of the mtDNA control region with the use of the primers ThrL16272 (ref. 28) and DLH 16750 (ref. 29). Sequences were aligned with published sequences of two morphs sampled from Queensland, Australia, and an outgroup (*R. arcuatus*)¹¹. We performed phylogenetic reconstruction by maximum parsimony analysis (heuristic search) in PAUP. We derived a 50% majority-rule consensus tree and assessed the reliability of clades by bootstrapping (1,000 iterations). Pairwise divergence between all sequences was calculated (HKY85 model).

Received 3 December 2003; accepted 11 March 2004; doi:10.1038/nature02487.

1. Schluter, D. Ecology and the origin of species. *Trends Ecol. Evol.* **16**, 372–380 (2001).
2. Via, S. Sympatric speciation in animals: the ugly duckling grows up. *Trends Ecol. Evol.* **16**, 381–390 (2001).
3. Rice, W. R. & Hostert, E. E. Perspective: Laboratory experiments on speciation: what have we learned in forty years? *Evolution* **47**, 1637–1653 (1993).
4. Guillén Servent, A., Francis, C. M. & Ricklefs, R. E. in *Horseshoe Bats of the World (Chiroptera: Rhinolophidae)* (eds Corsor, G., Ujhelyi, P. & Thomas, N) xii–xxiv (Alana Books, Bishop’s Castle, Shropshire, UK, 2003).
5. Bruns, V. Peripheral auditory tuning for fine frequency analysis by the CF-FM bat, *Rhinolophus ferrumequinum*. I. Mechanical specializations of the cochlea. *J. Comp. Physiol.* **106**, 77–86 (1976).
6. Schuller, G. & Pollak, G. D. Disproportionate frequency representation in the inferior colliculus of Doppler-compensating greater horseshoe bats, evidence for an acoustic fovea. *J. Comp. Physiol. A* **132**, 47–52 (1979).
7. Schnitzler, H.-U. in *Recent Advances in the Study of Bats* (eds Fenton, M. B., Racey, P. A. & Rayner, J. M. V.) 226–243 (Cambridge Univ. Press, 1987).
8. Hartley, D. J. & Suthers, R. A. The acoustics of the vocal tract in the horseshoe bat, *Rhinolophus hildebrandti*. *J. Acoust. Soc. Am.* **84**, 1201–1213 (1988).
9. Schluter, D. & Nagel, L. M. Parallel speciation by natural selection. *Am. Nat.* **146**, 292–301 (1995).
10. Simmons, N. B. in *Mammal Species of the World: A Taxonomic and Geographic Reference* (eds Wilson, D. E. & Reeder, D. M.) 3rd edn (Smithsonian Institution Press, Washington DC, in the press).
11. Cooper, S. J. B., Reardon, T. B. & Skilins, J. Molecular systematics of Australian rhinolophid bats (Chiroptera: Rhinolophidae). *Aust. J. Zool.* **46**, 203–220 (1998).
12. Pye, J. D. Is fidelity futile? The ‘true’ signal is illusory, especially with ultrasound. *Bioacoustics* **4**, 271–286 (1993).
13. Kober, R. & Schnitzler, H.-U. Information in sonar echoes of fluttering insects available for echolocating bats. *J. Acoust. Soc. Am.* **87**, 882–896 (1990).
14. Houston, R. D., Boonman, A. M. & Jones, G. in *Echolocation in Bats and Dolphins* (eds Thomas, J. A., Moss, C. F. & Vater, M.) 339–344 (Univ. of Chicago Press, 2004).

15. Lawrence, B. D. & Simmons, J. A. Measurements of atmospheric attenuation at ultrasonic frequencies and the significance for echolocation by bats. *J. Acoust. Soc. Am.* **71**, 585–590 (1982).
16. Jones, G. & Barlow, K. E. in *Echolocation in Bats and Dolphins* (eds Thomas, J. A., Moss, C. F. & Vater, M.) 345–349 (Univ. of Chicago Press, 2004).
17. NATO Advanced Study Institute & Möhres, F. P. *Cours d’Été OTAN sur les Systèmes Sonars Animaux: Biologie et Bionique* 2 939–945 (Laboratoire de Physiologie Acoustique, Paris, 1966).
18. Matsumura, S. Mother–infant communication in a horseshoe bat (*Rhinolophus ferrumequinum nippon*): vocal communication in three-week old infants. *J. Mamm.* **62**, 20–28 (1981).
19. Andrews, M. M. & Andrews, P. T. Ultrasound social calls made by greater horseshoe bats (*Rhinolophus ferrumequinum*) in a nursery roost. *Acta Chiropterol.* **5**, 221–234 (2003).
20. Long, G. R. & Schnitzler, H.-U. Behavioural audiograms from the bat, *Rhinolophus ferrumequinum*. *J. Comp. Physiol. A* **100**, 211–219 (1975).
21. Fenton, M. B. *Communication in the Chiroptera* (Indiana Univ. Press, 1985).
22. Francis, C. M. & Habersetzer, J. in *Bat Biology and Conservation* (eds Kunz, T. H. & Racey, P. A.) 169–181 (Smithsonian Institution Press, Washington DC, 1998).
23. Vater, M. in *Ontogeny, Functional Ecology and Evolution of Bats* (eds Adams, R. A. & Pedersen, S. C.) 137–173 (Cambridge Univ. Press, 2000).
24. Rübsamen, R. & Schäfer, M. Audio-vocal interactions during development? Vocalisation in deafened young horseshoe bats vs. audition in vocalization-impaired bats. *J. Comp. Physiol. A* **167**, 771–784 (1990).
25. Rossiter, S. J., Burland, T. M., Jones, G. & Barratt, E. M. Characterization of microsatellite loci in the greater horseshoe bat *Rhinolophus ferrumequinum*. *Mol. Ecol.* **8**, 1957–1969 (1999).
26. Dawson, D. A., Rossiter, S. J., Jones, G. & Faulkes, C. F. Microsatellite loci for the greater horseshoe bat, *Rhinolophus ferrumequinum* (Rhinolophidae, Chiroptera) and their cross-utility in 17 other bat species. *Mol. Ecol. Notes* **4**, 96–100 (2004).
27. Nichols, R. A., Bruford, M. W. & Groombridge, J. J. Sustaining genetic variation in a small population: evidence from the Mauritius kestrel. *Mol. Ecol.* **10**, 593–602 (2001).
28. Stanley, H. F. *et al.* Worldwide patterns of mitochondrial DNA differentiation in the harbour seal (*Phoca vitulina*). *Mol. Biol. Evol.* **13**, 368–382 (1996).
29. Wilkinson, G. S. & Chapman, A. M. Length and sequence variation in Evening Bat D-Loop mtDNA. *Genetics* **128**, 607–617 (1991).
30. Churchill, S. *Australian Bats* (New Holland, Sydney, 1998).

Supplementary Information accompanies the paper on www.nature.com/nature.

Acknowledgements We thank the Indonesian Institute of Science (LIPI) and the Wallacea Development Institute for granting permissions to undertake this work, and T. Coles, Boedi and Operation Wallacea staff and volunteers for logistical support in Indonesia. We thank Irman and Samsudin for help in the field; A. Boonman, T. Burland, D. Dawson, C. Faulkes, K. Freiman, B. Kirsten, R. Nichols, L. Pettersson and J. Storz for advice on analysis and technical support; and B. Fenton and G. Jones for helpful comments on the manuscript. This work was funded by Operation Wallacea, and microsatellite development was supported by the NERC-funded Sheffield Molecular Genetics Facility.

Authors’ contributions T.K. and S.J.R. performed the acoustic and genetic analyses, respectively, and jointly undertook the fieldwork and wrote the paper. The order of authors is alphabetical.

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

Correspondence and requests for materials should be addressed to T.K. (tigga@bu.edu). Accession numbers for new and published sequences are AY568637–AY568646, and AF065069–AF065073 and AF065090 (ref. 12), respectively.

Echolocation signals reflect niche differentiation in five sympatric congeneric bat species

Björn M. Siemers & Hans-Ulrich Schnitzler

Animal Physiology, Zoological Institute, University of Tübingen, Morgenstelle 28, 72076 Tübingen, Germany

Echolocating bats can be divided into guilds according to their preferred habitat and foraging behaviour^{1–4}, which coincide with distinct adaptations in wing morphology⁵ and structure of echolocation signals⁶. Although coarse structuring of niche space between different guilds is generally accepted, it is not clear how niches differ within guilds^{7–10}, or whether there is fine-grained niche differentiation reflected in echolocation signal structure^{11,12}. Using a standardized performance test, here we show clutter-dependent differences in prey-capture success for