

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).

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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).

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Echolocation signals reflect niche differentiation in five sympatric congeneric bat species

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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

bats from five species of European *Myotis*. These species are morphologically similar, sympatric¹³, and all belong to the guild labelled “edge space aerial/trawling foragers”⁴. We further demonstrate a strong correlation between the prey-detection ability of the species and the respective search-call bandwidth. Our findings indicate that differences in echolocation signals contribute to within-guild niche differentiation. This is the first study relating sensory abilities of a set of potentially competing animal species to a direct measure of their respective foraging performance, suggesting an important role of sensory ecology in the structuring of animal communities.

Each of the five bat species catches flying insects or spiders on threads close to the clutter-producing foliage of vegetation^{13–18}, so they are all faced with the problem of distinguishing echoes from prey and from background or clutter^{2–4,6}. Two species also take insects from flat water surfaces by trawling, and thus have to cope with clutter echoes from drifting targets or the shore^{14,17}. Using previously published field data on foraging behaviour, diet and microhabitat use^{13–19} as well as morphometric data²⁰, we hypothesized that the five species would differ in ability to reject clutter. This, in turn, should be reflected in the minimal distance from background at which the bats could find prey by echolocation. From these data, we predicted the following order of decreasing prey-capture ability: *M. nattereri*, *M. emarginatus*, *M. mystacinus*, *M. daubentonii*, *M. dasycneme*. To test this prediction, we encouraged all species to catch prey as close as possible to a standardized clutter-producing background and measured their minimal capture distances (defined as the distance corresponding to 50% capture success).

In a flight tent, we presented mealworms to freshly captured bats at different distances to a vertical ‘clutter screen’ (polypropylene carpet with many latex-clay nubs; see Methods) that produced clutter echoes. In the dark, all bats from all of the five species located and captured silent, motionless prey close to the clutter screen. Bats also attacked rubber prey dummies lacking the odour of prey (total of 359 attacks on dummies: 39 by *M. nattereri*, 81 by *M. emarginatus*, 101 by *M. mystacinus*, 138 by *M. daubentonii*; dummies not presented to *M. dasycneme*). We conclude that visual, olfactory and passive acoustic cues were not necessary for prey detection, location and capture, all of which could be accomplished by echolocation alone. This is further corroborated by the fact that the bats of all five species consistently produced approach sequences that terminated with a ‘buzz’ (a series of very short calls with a repetition rate of about 200 Hz) typical of aerial hawking bats³. In our experiments, no bat ever captured a mealworm that was presented directly on the clutter background (Fig. 1). As in previous studies^{18,21,22}, this finding suggests that bats could not use echolocation to distinguish prey directly positioned on clutter-producing background. At distances of 25 and 50 cm almost all mealworms were captured by all bats of all five species. However, capture success differed significantly between species when prey were presented at 5 and 10 cm from clutter (Table 1) and decreased in the order of species we had predicted (Fig. 1). These differences in prey-capture ability suggest that some within-guild structuring of niche space exists.

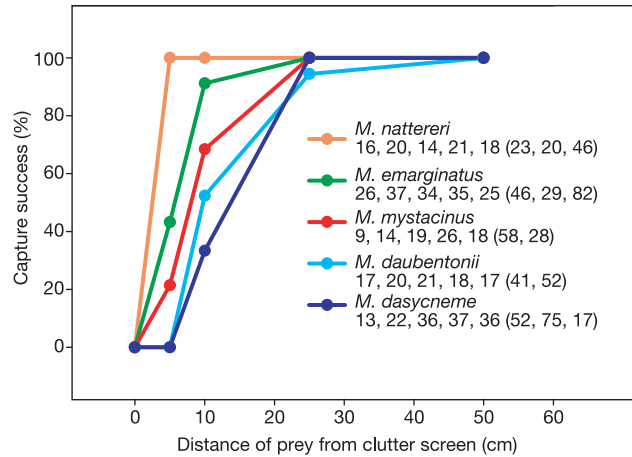


Figure 1 The capture performance of bats from five sympatric *Myotis* species searching for prey offered at different distances from a clutter screen that mimicked a vegetation edge. Species’ abilities differed at distances of 5 and 10 cm (see Table 1 for statistics). For this graph, individual performances were pooled for each species (for individual performance data see Supplementary Figure). Trials per species per distance of 0, 5, 10, 25 and 50 cm are given below the species names (parentheses enclose trials per individual). We performed a total of 569 trials.

Parameters of wing morphology, such as low wing loading or low aspect ratio, are related causally to the ability to sustain slow, manoeuvrable flight⁵, a prerequisite for consistently capturing prey close to vegetation or a clutter screen. However, although the wing loading is nearly identical in *M. emarginatus*, *M. mystacinus* and *M. daubentonii*⁵ and they indeed showed similar flight and approach behaviour in our experiments, their capture performance differed considerably. Therefore, we argue that this measured difference in capture success was caused mainly by differences in echolocation call parameters and related differences in echo-processing capabilities. To test this hypothesis we compared the measured minimal capture distances with a number of signal parameters.

The search calls used in our standardized experimental situation were steep broadband frequency-modulated (FM) signals and averaged 1.4–1.8 ms in duration (Fig. 2). The first harmonic was always most prominent. Starting, peak and terminal frequency, bandwidth and pulse interval differed between the species, whereas pulse duration showed no significant differences between the five congeners (Table 2). Capture performance was unrelated to pulse duration, peak frequency or terminal frequency (Table 2). Species with higher starting frequencies and bandwidths and shorter pulse intervals were able to capture prey closer to clutter than those with lower starting frequencies and bandwidths and higher pulse intervals (Table 2, Fig. 3). We qualitatively and quantitatively corroborated the link between bandwidth and performance by building a successful logistic model (see Supplementary Methods) with the individual performance data (see Supplementary Figure). We further examined

Table 1 Capture performance comparison for five sympatric *Myotis* species

Distance of prey from the clutter screen (cm)	Species	<i>M. emarginatus</i>	<i>M. mystacinus</i>	<i>M. daubentonii</i>	<i>M. dasycneme</i>
5	<i>M. nattereri</i>	***	***	***	***
	<i>M. emarginatus</i>		NS	**	**
	<i>M. mystacinus</i>			(*)	(*)
	<i>M. daubentonii</i>			*	NS
	<i>M. nattereri</i>	NS	(*)	*	***
10	<i>M. emarginatus</i>		(*)	*	***
	<i>M. mystacinus</i>			NS	(*)
	<i>M. daubentonii</i>				NS
	<i>M. nattereri</i>				

NS, not significant (probability $P > 0.05$); (*), significance lost when Bonferroni correction is applied; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

For each pair-wise comparison, all successful and unsuccessful trials of the two species were cross-tabulated and subjected to χ^2 test (degrees of freedom, 1). The P values tabulated here are Bonferroni-corrected to account for the multiple comparisons (ten comparisons per distance gives P values $\times 10$).

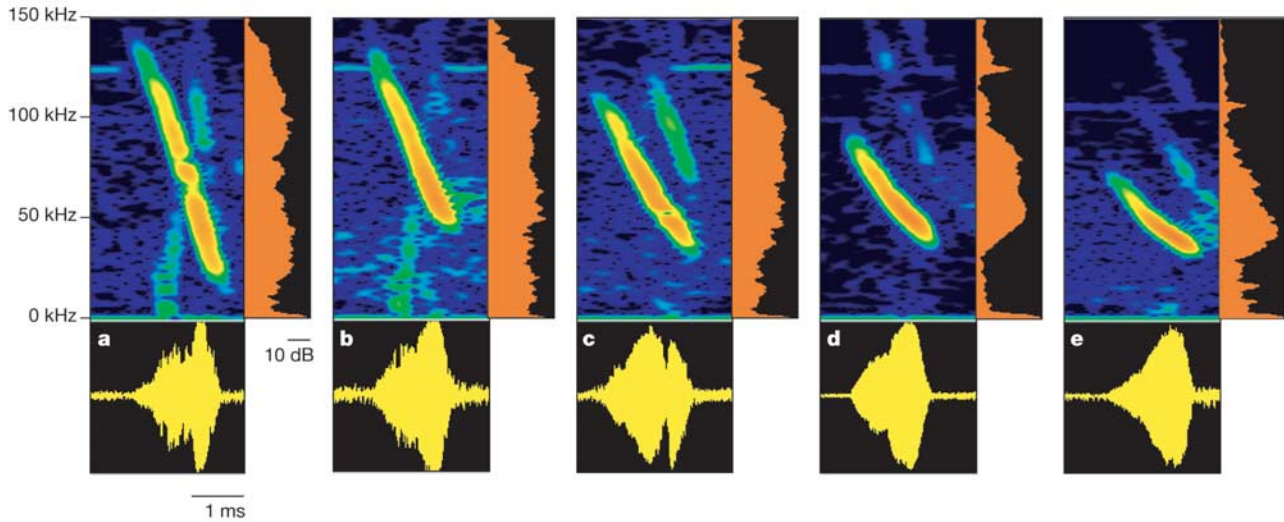


Figure 2 Representative search calls of five sympatric *Myotis* species in sonogram representation with time signal below and averaged power spectrum on the right. **a**, *Myotis nattereri*; **b**, *M. emarginatus*; **c**, *M. mystacinus*; **d**, *M. daubentonii*; and

e, *M. dasycneme*. See Methods for definition of search calls. Notches in signals (**a**, **c**) are caused by two-wavefront interference.

whether this correlation was a mere product of phylogenetic inertia by performing an independent contrast regression analysis²³ using a recent molecular phylogeny of the genus *Myotis*²⁴. Apparently, the association of broad bandwidth and good capture ability has evolved several times convergently even among the species examined, as there are two clades including a high-bandwidth species with good capture abilities and a low-bandwidth species with lower abilities (an *M. nattereri*–*M. daubentonii* clade and an *M. emarginatus*–*M. dasycneme* clade). Consequently, the correlation between bandwidth and minimal capture distance remained strong and significant for the phylogenetically corrected data set ($R^2 = 0.95$, $P = 0.0047$), with a minimal capture distance contrast slope of -0.14 with respect to contrast in bandwidth, strikingly similar to that in the non-corrected data (see Fig. 3).

When comparing the four species with low to medium capture ability, it became evident that the broadening of the bandwidth was achieved mainly by an increase in the starting frequency. The very high bandwidth of the best-performing species *M. nattereri* resulted from an additional lowering of the terminal frequency (Table 2). This may indicate that not only absolute frequency, but especially bandwidth is a crucial parameter for the improvement of prey-detection ability.

It could be argued that the bandwidth-dependent increase in range accuracy and range resolution helps bats to find prey²⁵. This explanation might be considered if the search flights were perpen-

dicular to the clutter screen, which would keep the prey echoes in front of the clutter echoes. However, the bats chose an oblique flight path and because of the width of the sonar footprint, the signals hit the screen before they hit the prey and therefore the prey echoes were buried in clutter. Hence, our experimental setting did not constitute a simple ranging task. Rather, we believe that the bats were faced with the classification problem of discriminating clutter echoes without and with prey echo. Our data show that all bats tolerated some overlap of prey echoes and background echoes.

However, until now no convincing hypothesis has been proposed to explain how bats can recognize a desired target amongst clutter, except for flower-visiting bats that use conspicuous echoes provided by the plants they pollinate²⁶. Our data may help to increase the understanding of how bats solve such complex classification problems, because they suggest that high starting frequencies and large bandwidths for good performance in such a task have adaptive value. The width of the sonar footprint on the clutter screen is reduced with increasing frequency, because of the higher directionality of the sonar beam that decreases the number of echoes from clutter at short distances. Therefore, using high-frequency signals could be a good strategy. In addition to an evolutionary increase in starting frequency, bats could achieve a behavioural reduction of the sonar footprint by keeping the search distance short²⁷. To investigate whether bats improve spatial unmasking by optimizing flight

Table 2 Search call parameters and their correlation with minimal capture distances

	Starting frequency (kHz)	Peak frequency (kHz)	Terminal frequency (kHz)	Bandwidth (kHz)	Pulse duration (ms)	Pulse interval (ms)
<i>M. nattereri</i> (3 IU, 63 calls)	135.4 ± 2.7	43.0 ± 4.1	16.2 ± 0.5	119.2 ± 2.5	1.4 ± 0.1	46.4 ± 8.9
<i>M. emarginatus</i> (3 IU, 153 calls)	133.5 ± 8.0	58.7 ± 1.6	42.2 ± 1.1	91.2 ± 8.4	1.7 ± 0.1	45.1 ± 1.8
<i>M. mystacinus</i> (2 bats, 111 calls)	107.0 ± 2.5	50.2 ± 0.5	31.6 ± 0.8	75.4 ± 3.3	1.7 ± 0.1	55.5 ± 0.9
<i>M. daubentonii</i> (2 bats, 171 calls)	90.1 ± 4.0	51.6 ± 0.5	32.9 ± 1.5	57.2 ± 2.5	1.8 ± 0.1	51.2 ± 0.2
<i>M. dasycneme</i> (3 bats, 172 calls)	73.2 ± 1.7	40.2 ± 1.1	29.4 ± 1.0	43.7 ± 2.5	1.7 ± 0.1	64.7 ± 6.6
ANOVA of call parameters (d.f. = 4)						
F-ratio	100.2	29.9	264.4	109.7	0.9	5.9
Probability, P	<0.0001	<0.0001	<0.0001	<0.0001	0.5210, NS	0.0164
Regression of minimal capture distance on call parameters (d.f. = 1)						
R ²	0.96	0.12	0.06	0.95	0.39	0.81
F-ratio	72.2	0.4	0.2	63.0	1.9	12.8
P	0.0034	0.5719, NS	0.6889, NS	0.0042	0.2602, NS	0.037

s.d., Standard deviation; d.f., degrees of freedom.

Values are shown as mean ± s.d. We used individual means to compare the call parameters between the species: 9 to 104 calls from 3 to 7 sequences for each individual (total of 670 calls). Statistical data is given under analysis of variance (ANOVA); second-order means are tabulated. To analyse the influence of the call parameters on prey-capture ability, we calculated a linear regression of minimal capture distance (species means, for data and definition see Fig. 3) on each of the call parameters (species means, see regression data).

behaviour and call directionality during both search and approach, further studies with three-dimensional analysis of flight-path and sonar-beam characteristics will be required.

We believe that an increase in bandwidth is probably advantageous because of the illumination of the sonar scene with a wider range of wavelengths: there is an 8.4-fold decrease from starting to terminal frequency in the case of the best-performing *M. nattereri*. An *M. nattereri* call that sweeps from 135 to 16 kHz contains wavelengths from 2.6 to 21.8 mm. Many arthropods (including the mealworms we used) and many reflecting background facets (leaves, grass tips and also the nubs of our clutter screen) fall within this size range. With a broadband call, many reflectors will be illuminated with wavelengths above, at and below their sizes simultaneously. Echo intensity, directionality and other reflection properties depend strongly on whether the wavelength of the incident sound is above, well below or about the same as the reflector size²⁸. Therefore, a given sonar scene may convey clear differences in the information content at frequency channels which are far apart. Bats using broadband calls presumably sample frequency-dependent differences efficiently and develop a detailed characterization of the background contours necessary for a better separation of prey from background.

Gleaning bats from the families Megadermatidae and Phyllostomidae produce broad bandwidths by using multiharmonic signals and often, but not always, rely on passive acoustic prey detection^{2,3,27}. The *Myotis* in our study as well as the palaeotropical Kerivoulinae and Murinae²⁹ (all family Vespertilionidae) achieve large call bandwidths with the first harmonic only, which may possibly prove to be a key innovation for prey detection by echolocation close to clutter.

Our data suggest that the differences in prey-detection abilities of different bats even within groups of morphologically and ecologically similar species are linked to differences in their echolocation signals and associated sensory abilities. Our data further indicate that differences in sensory abilities generate differences in prey availability for different bat species, even when foraging in exactly the same place. Therefore, differences in echolocation signals and associated sensory abilities contribute to within-guild resource partitioning. From our findings in bats, we suggest that the sensory ecologies of potentially competing species might play an important role in the structuring of animal communities. □

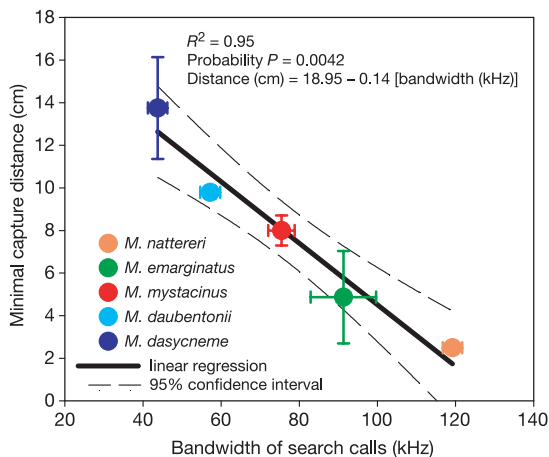


Figure 3 Correlation of 'minimal capture distance' with the bandwidth of search calls for the five sympatric *Myotis* species. We defined minimal capture distance as the distance of prey to the clutter screen that corresponded to 50% capture success. Minimal capture distance was calculated for every individual by linear interpolation from the two neighbouring distances for which performance was measured (for individual performance data see Supplementary Figure). Data are plotted as species means and error bars give the standard deviation. For number of bats see Methods.

Methods

Animals and flight tent

Bats were captured in Germany under licence from the responsible authorities (BR Hannover licence no. 503.41-22201/3; RP Karlsruhe 73c1-8852.15; RP Tübingen 73-8/8852.21; RP Stuttgart 73-8850.68-14/ Uni Tübingen; RP Freiburg Az: 73/8852.46-2) and released to the wild at the site of capture following completion of experiments. The freshly captured, experimentally naive bats were released into a mobile flight tent with a ground area of 3.5 by 7 m and about 2.5 m height, erected close to the site where the bats were captured. It had a natural light regime. We present data from three *M. dasycneme* (male), two *M. daubentonii* (male), eight *M. emarginatus* (female), two *M. mystacinus* (one male, one female) and four *M. nattereri* (one male, three female).

Behavioural experiments

Experiments were run during the natural activity period of the bats, either in complete darkness (moonless night) or with dim light (artificial or moonlight) from outside the flight tent. To assess the capture ability of the different species close to clutter-producing background, one mealworm (*Tenebrio molitor* Coleoptera) at a time was suspended on a thread (of diameter 0.1 mm) 0, 5, 10, 25 and 50 cm in front of a vertical clutter screen. A trial was scored as successful if the bat found and captured the mealworm within a 1-min time window of continuous search flight. We continuously modified the position of the mealworms in front of the clutter screen (left–right, up–down) to avoid conditioning the bats to a certain location of the screen and pseudo-randomly alternated between distances within any one session. It is important to note that we examined the bats in rather an unnatural situation, and so we did not necessarily measure the minimal detection distances bats will show in the wild, when searching for insects in front of vegetation. In natural situations, the classification problem of discriminating clutter echoes without and with prey echo will be far more complex because vegetation contours will be more irregular than our clutter screen and will deliver clutter echoes not only from the outer contour but also from the reflecting facets below³⁰.

We performed the experiments separately with only one bat flying at a time, with the exception of two *M. nattereri*, and three and four *M. emarginatus* that had to be flown together for motivational reasons. Their performances and echolocation calls were combined to form one 'individual unit' (IU) each. Thus we have presented data for 13 individuals (bats or IUs, respectively).

We used a vertical clutter screen to mimic vegetation edges in a standardized way. It consisted of a polypropylene carpet (208 cm wide, 170 cm high) with latex-clay half-spheres (nubs) of 5-mm diameter in a regular pattern with 12-mm spacing between them. When ensonified at an acute angle with a bat-like FM pulse, it returned many overlapping copies of this signal²², which is an echo complex somewhat similar to the echoes of natural vegetation³⁰.

To investigate the importance of arthropod-specific cues for prey detection, rubber dummies (electrical shrinkwrap tubing ranging from 1.6 to 12.7 mm in diameter and 1 to 47 mm in length) were offered to the bats by suspending them on nylon threads in the flight tent.

Search call recordings and sound analysis

Sequences of echolocation calls were recorded from each bat when flying in front of the clutter screen during trials in which they did not find the mealworm. We assumed that the bats were searching for (and not approaching) prey and we therefore considered these echolocation signals to be search calls. Sound recording and analysis were performed with custom-built hard- and software as described in detail elsewhere¹⁸ (256 FFT, 93.75% overlap). Sound duration and pulse interval were measured from the time signal. Starting frequency and terminal frequency were determined from a sonagram representation at 25 dB below peak frequency (that is, frequency with maximum amplitude) on the first harmonic of each signal. The –25 dB bandwidth (that is, sweep range) of each signal was computed as starting frequency minus terminal frequency.

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- Aldridge, H. D. J. N. & Rautenbach, I. L. Morphology, echolocation and resource partitioning in insectivorous bats. *J. Anim. Ecol.* **56**, 763–778 (1987).
- Neuweiler, G. Auditory adaptations for prey capture in echolocating bats. *Physiol. Rev.* **70**, 615–641 (1990).
- Schnitzler, H.-U. & Kalko, E. K. V. Echolocation by insect-eating bats. *Bioscience* **51**, 557–569 (2001).
- Schnitzler, H.-U., Moss, C. F. & Denzinger, A. From spatial orientation to food acquisition in echolocating bats. *Trends Ecol. Evol.* **18**, 386–394 (2003).
- Norberg, U. M. & Rayner, J. M. V. Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Phil. Trans. R. Soc. Lond. B* **316**, 335–427 (1987).
- Fenton, M. B. Foraging behaviour and ecology of animal-eating bats. *Can. J. Zool.* **68**, 411–422 (1990).
- Findley, J. S. The structure of bat communities. *Am. Nat.* **110**, 129–139 (1976).
- McKenzie, N. L. & Rolfe, J. K. Structure of bat guilds in the Kimberley mangroves, Australia. *J. Anim. Ecol.* **55**, 401–420 (1986).
- Crome, F. H. J. & Richards, G. C. Bats and gaps: microchiropteran community structure in a Queensland rain forest. *Ecology* **69**, 1960–1969 (1988).
- Sleep, D. J. H. & Brigham, R. M. An experimental test of clutter tolerance in bats. *J. Mamm.* **84**, 216–224 (2003).
- Heller, K.-G. & von Helversen, O. Resource partitioning of sonar frequency bands in rhinolophoid bats. *Oecologia* **80**, 178–186 (1989).
- Kingston, T., Jones, G., Zubaid, A. & Kunz, T. H. Resource partitioning in rhinolophoid bats revisited. *Oecologia* **124**, 332–342 (2000).
- Krapp, F. in *Handbuch der Säugetiere Europas* Vol. 4/1 (eds Niethammer, J. & Krapp, F.) 257–442 (Aula, Wiebelsheim, 2001).

14. Jones, G. & Rayner, J. M. V. Flight performance, foraging tactics and echolocation in free-living Daubenton's bats *Myotis daubentonii* (Chiroptera: Vespertilionidae). *J. Zool.* **215**, 113–132 (1988).
15. Krull, D., Schumm, A., Metzner, W. & Neuweiler, G. Foraging areas and foraging behavior in the notch-eared bat, *Myotis emarginatus* (Vespertilionidae). *Behav. Ecol. Sociobiol.* **28**, 247–253 (1991).
16. Schumm, A., Krull, D. & Neuweiler, G. Echolocation in the notch-eared bat, *Myotis emarginatus*. *Behav. Ecol. Sociobiol.* **28**, 255–261 (1991).
17. Britton, A. R. C., Jones, G., Rayner, J. M. V., Boonman, A. M. & Verboom, B. Flight performance, echolocation and foraging behaviour in pond bats, *Myotis dasycneme* (Chiroptera: Vespertilionidae). *J. Zool.* **241**, 503–522 (1997).
18. Siemers, B. M. & Schnitzler, H.-U. Natterer's bat (*Myotis nattereri* Kuhl, 1818) hawks for prey close to vegetation using echolocation signals of very broad bandwidth. *Behav. Ecol. Sociobiol.* **47**, 400–412 (2000).
19. Swift, S. M. & Racey, P. A. Gleaning as a foraging strategy in Natterer's bat *Myotis nattereri*. *Behav. Ecol. Sociobiol.* **52**, 408–416 (2002).
20. Fenton, M. B. & Bogdanowicz, W. Relationships between external morphology and foraging behaviour: bats in the genus *Myotis*. *Can. J. Zool.* **80**, 1004–1013 (2002).
21. Arlettaz, R., Jones, G. & Racey, P. A. Effect of acoustic clutter on prey detection by bats. *Nature* **414**, 742–745 (2001).
22. Siemers, B. M., Stitz, P. & Schnitzler, H.-U. The acoustic advantage of hunting at low heights above water: behavioural experiments on the European 'trawling' bats *Myotis capaccinii*, *M. dasycneme* and *M. daubentonii*. *J. Exp. Biol.* **204**, 3843–3854 (2001).
23. Harvey, P. H. & Pagel, M. D. *The Comparative Method in Evolutionary Biology* 138–162 (Oxford Univ. Press, Oxford, 1991).
24. Ruedi, M. & Mayer, F. Molecular systematics of bats of the genus *Myotis* (Vespertilionidae) suggests deterministic ecomorphological convergences. *Mol. Phylog. Evol.* **21**, 436–448 (2001).
25. Simmons, J. A. & Stein, R. A. Acoustic imaging in bat sonar: Echolocation signals and the evolution of echolocation. *J. Comp. Physiol. A* **135**, 61–84 (1980).
26. von Helversen, O. & von Helversen, D. Acoustic guide in bat pollinated flower. *Nature* **398**, 759–760 (1999).
27. Schmidt, S., Hanke, S. & Pillat, J. The role of echolocation in the hunting of terrestrial prey—new evidence for an underestimated strategy in the gleaning bat, *Megaderma lyra*. *J. Comp. Physiol. A* **186**, 975–988 (2000).
28. Morse, P. M. & Ingard, K. U. *Theoretical Acoustics* (Princeton Univ. Press, Princeton, 1986).
29. Kingston, T., Jones, G., Akbar, Z. & Kunz, T. H. Echolocation signal design in Kerivoulineae and Murinae (Chiroptera: Vespertilionidae) from Malaysia. *J. Zool.* **249**, 359–374 (1999).
30. Müller, R. & Kuc, R. Foliage echoes: A probe into the ecological acoustics of bat echolocation. *J. Acoust. Soc. Am.* **108**, 836–845 (2000).

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Metabolic network analysis of the causes and evolution of enzyme dispensability in yeast

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Under laboratory conditions 80% of yeast genes seem not to be essential for viability¹. This raises the question of what the mechanistic basis for dispensability is, and whether it is the result of selection for buffering or an incidental side product. Here we analyse these issues using an *in silico* flux model^{2–5} of the yeast metabolic network. The model correctly predicts the knock-out fitness effects in 88% of the genes studied⁴ and *in vivo* fluxes.

Dispensable genes might be important, but under conditions not yet examined in the laboratory. Our model indicates that this is the dominant explanation for apparent dispensability, accounting for 37–68% of dispensable genes, whereas 15–28% of them are compensated by a duplicate, and only 4–17% are buffered by metabolic network flux reorganization. For over one-half of those not important under nutrient-rich conditions, we can predict conditions when they will be important. As expected, such condition-specific genes have a more restricted phylogenetic distribution. Gene duplicates catalysing the same reaction are not more common for indispensable reactions, suggesting that the reason for their retention is not to provide compensation. Instead their presence is better explained by selection for high enzymatic flux.

Although many single-gene deletions have negligible effects on growth rates under laboratory conditions^{1,6}, the causes and evolution of gene dispensability has remained a controversial issue^{7–9}. The capacity of organisms to compensate mutations partly stems from gene duplicates⁸, whereas alternative metabolic pathways might also have a role^{7,10–12}. The one previous systematic analysis on a eukaryotic organism¹³ used a gene's rate of evolution as a proxy for dispensability, a supposition now considered questionable¹⁴. A third possibility, and one that has received relatively little attention, is that genes only seem to be non-essential, and that they have important roles under environmental conditions yet to be replicated in the laboratory^{8,15}.

To investigate the causes of gene dispensability, the metabolic capabilities of the *Saccharomyces cerevisiae* network were calculated using flux balance analysis (FBA)¹⁶. The previously reconstructed network^{2,4} consists of 809 metabolites as nodes (including external metabolites), connected by 851 different biochemical reactions (including transport processes). The method first defines a solution space of fluxes of all metabolic reactions in the network that satisfy the governing constraints (that is, steady state of metabolites, flux capacity, direction of reactions, nutrients available in the environments; see Methods). Next, the optimal use of the metabolic network to produce major biosynthetic components for growth can be found among all possible solutions using various optimization protocols^{3,4}. The FBA and MOMA⁵ (minimization of metabolic adjustment) protocols enable us to predict the phenotypic behaviour of nutritional changes and gene deletions, along with the concomitant changes in flux distributions.

We start by asking how well the mathematical model predicts experimentally measured fluxes, and the effects of gene deletions. We then use it to address the relative importance of the suggested mechanisms for gene dispensability. Finally, we ask whether dispensability is a directly selected feature or a side consequence.

Owing to the availability of systematic knockout studies¹ and some experimentally measured fluxes under four different growth conditions¹⁷, we can directly test the predictive power of the mathematical protocol. We initiated the model to mimic the growth conditions used in these experimental studies. The model correctly predicts: (1) relative differences in flux values; (2) presence or absence of fluxes in 91–95% of the cases; (3) the fitness effects of 88% of single-gene deletions under nutrient-rich growth conditions⁴ (see Supplementary Tables S1–S3). Although the model ignores details of gene regulation, the predicted variations in the activity of metabolic pathways across environments are consistent with observations (Supplementary Tables S1 and S2; see also ref. 3). The method, although robust, is not perfect. Although the frequency of experimentally verified essential genes in the group of genes with zero predicted flux is low on rich medium, it is not zero (8.8% for genes with zero flux compared with 28.8% for the rest; $\chi^2 = 18.54$, $P < 10^{-4}$, 1 degree of freedom (d.f.)). The few essential genes in this group probably represent incomplete biochemical knowledge, missing components from the biomass equation, or pleiotropic gene functions⁴.