

**Figure 4** A comparison of the locations of tomographic velocity anomalies and the expected palaeolocations of the Siberian active margins. **a**, Locations of the lithospheric suture and the main axis of the ocean-lithospheric slab remnants as a function of depth, as determined from our tomographic results. **b**, Present-day and palaeogeographically reconstructed locations of the Mongol-Okhotsk-Verkhoyansk suture zones as a function of time (longitudes are arbitrary), using the palaeomagnetic pole determinations for Siberia of Zhao *et al.*<sup>29</sup> (81° N, 158.6° E for 50 Myr; 73.8° N, 202.4° E for 85–120 Myr; 70.1° N, 184.3° E for 150 Myr).

into the lower mantle even after subduction stopped some 150 Myr ago. Whether this visibility is a result of temperature<sup>26</sup>, composition, pressure, or a combination thereof, is an open question, but what is clear is that the Mongol-Okhotsk subducted lithospheric material has been feeding the 'graveyard' of slabs under Asia. Our conclusions also imply that significant downwelling is a characteristic of growing supercontinents for hundred of millions of years<sup>27</sup>, and that most, if not all, significantly fast anomalies in the deeper mantle appear to be associated with past subduction. This renders tomography an important tool for testing palaeogeographical reconstructions. □

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1. van der Hilst, R. D., Widiyantoro, S. & Engdahl, E. R. Evidence for deep-mantle circulation from global tomography. *Nature* **386**, 578–584 (1997).
2. Grand, S., van der Hilst, R. D. & Widiyantoro, S. Global seismic tomography: A snapshot of convection in the Earth. *GSA today* **7**, 1–7 (1997).
3. van der Hilst, R., Engdahl, R., Spakman, W. & Nolet, G. Tomographic imaging of subducted lithosphere below the northwest Pacific island arcs. *Nature* **353**, 37–43 (1991).
4. Richards, M. A. & Engebretson, D. C. Large-scale mantle convection and the history of subduction. *Nature* **355**, 437–440 (1992).
5. Wen, L. & Anderson, D. L. The fate of slabs inferred from seismic tomography and 130 million years of subduction. *Earth Planet. Sci. Lett.* **133**, 185–198 (1995).
6. Şengör, A. M. C. & Natal'in, B. A. in *The Tectonic Evolution of Asia* (eds Yin, A. & Harrison, M.) 486–640 (Cambridge Univ. Press, 1996).
7. Zhao, X. X., Coe, R. S., Zhou, Y., Wu, H. & Wang, J. New paleomagnetic results from northern China: Collision and suturing with Siberia and Kazakhstan. *Tectonophysics* **181**, 43–81 (1990).
8. Enkin, R., Yang, Z. Y., Chen, Y. & Courtillot, V. Paleomagnetic constraints on the geodynamic history of the major blocks of China from the Permian to the Present. *J. Geophys. Res.* **97**, 13953–13989 (1992).
9. Wyssession, M. E. in *Subduction* (eds Bebout, G. *et al.*) 369–384 (Monogr. 96, Am. Geophys. Union, Washington DC, 1996).
10. Yin, A. & Nie, S. Y. in *The Tectonic Evolution of Asia* (eds Yin, A. & Harrison, M.) 442–485 (Cambridge Univ. Press, 1996).
11. Gilder, S. & Courtillot, V. Timing of the North-South China collision from new middle to late Mesozoic paleomagnetic data from the North China block. *J. Geophys. Res.* **102**, 17713–17727 (1997).
12. Ziegler, A. M. *et al.* in *The Tectonic Evolution of Asia* (eds Yin, A. & Harrison, M.) 371–400 (Cambridge Univ. Press, 1996).
13. Parfenov, L. M. Tectonics of the Verkhoyansk-Kolyma Mesozoides in the context of plate tectonics. *Tectonophysics* **199**, 319–342 (1991).

14. Bijwaard, H., Spakman, W. & Engdahl, R. Closing the gap between regional and global travel time tomography. *J. Geophys. Res.* (in the press).
15. Dziewonski, A. M., Hager, B. H. & O'Connell, R. J. Large-scale heterogeneities in the lower mantle. *J. Geophys. Res.* **82**, 239–255 (1977).
16. Zhou, H. A high resolution P-wave model for the top 1200 km of the mantle. *J. Geophys. Res.* **101**, 27791–27810 (1996).
17. Engdahl, E. R., van der Hilst, R. D. & Buland, R. P. Global teleseismic earthquake relocation with improved travel times and procedures for depth determination. *Bull. Seismol. Soc. Am.* **88**, 722–743 (1998).
18. Spakman, W. & Bijwaard, H. Irregular cell parameterization of tomographic problems. *Ann. Geophys.* **16** (suppl. 1), 28 (1998).
19. Curtis, C., Dost, B., Trampert, J. & Snieder, R. Eurasian fundamental mode surface wave phase velocities and their relationship with tectonic structures. *J. Geophys. Res.* (in the press).
20. Ritzwoller, M. H. & Levshin, A. L. Eurasian surface wave tomography: Group velocities. *J. Geophys. Res.* **103**, 4839–4878 (1998).
21. Cande, S. C., Raymond, C. A., Stock, J. & Haxby, W. F. Geophysics of the Pitman Fracture Zone and Pacific-Antarctic plate motions during the Cenozoic. *Science* **270**, 947–953 (1995).
22. Tarduno, J. A. & Cottrell, R. D. Paleomagnetic evidence for motion of the Hawaiian hotspot during formation of the Emperor Seamounts. *Earth Planet. Sci. Lett.* **153**, 171–180 (1997).
23. Ricard, Y., Richards, M. A., Lithgow-Bertelloni, C. & Le Stunff, Y. A geodynamic model of mantle density heterogeneity. *J. Geophys. Res.* **98**, 21895–21909 (1993).
24. Lithgow-Bertelloni, C. & Richards, M. A. The dynamics of Cenozoic and Mesozoic plate motions. *Rev. Geophysics* **36**, 27–78 (1998).
25. Scotese, C. R. & Golonka, J. *Paleomap Paleogeographic Atlas* (Paleomap project, Univ. Texas, Arlington, 1992).
26. Zhong, S. & Gurnis, M. Mantle convection with plates and mobile, faulted plate margins. *Science* **267**, 838–843 (1995).
27. Gurnis, M. Large-scale mantle convection and the aggregation and dispersal of supercontinents. *Nature* **232**, 695–699 (1988).
28. Kennett, B. L. N., Engdahl, E. R. & Buland, R. Constraints on seismic velocities in the Earth from travel times. *Geophys. J. Int.* **122**, 108–124 (1995).
29. Zhao, X. X., Coe, R. S., Gilder, S. A. & Frost, G. M. Palaeomagnetic constraints on palaeogeography of China: Implications for Gondwanaland. *Austr. J. Earth Sci.* **43**, 643–672 (1996).

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## Can aposematic signals evolve by gradual change?

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**Aposematic species, which signal conspicuously of their unprofitability to predators, have puzzled evolutionary biologists for over a century<sup>1,2</sup>. Although conspicuousness of unpalatable prey improves avoidance learning by predators<sup>3–5</sup>, it also involves an evolutionary paradox: with increasing detectability<sup>4,6–8</sup> the deviant aposematic prey would suffer high predation initially from naive predators. Here we test a neglected idea<sup>7–11</sup> that aposematic coloration may evolve by gradual change rather than by major mutations. Weak signals did not suffer high initial predation, but predators (great tits, *Parus major*) did not learn to separate them from cryptic palatable prey. Furthermore, enhanced avoidance of more conspicuous signals occurred only if predators had previously encountered relatively strong signals. Thus, the gradual-change hypothesis does not provide an easy solution to the initial evolution of aposematism through predator learning. However, the possibility remains that cost-free step-wise mutations over the range of weak signals could accumulate under neutral selection to produce effective strong signals.**

It has been assumed that sudden, pronounced mutations turned unpalatable cryptic prey into highly conspicuous forms. This hypothesis of sudden change may account for why grouping has been suggested to be essential in explaining how brightly coloured insects first evolved<sup>12–14</sup>. Naive predators encountering a group are likely to leave some of the unpalatable prey untouched, whereas any single solitary individual may not escape being predated. Alternatively, gradual change in prey detectability<sup>7–9,11</sup>, initially not changing the cryptic type into an overtly conspicuous prey type, may allow evolution of aposematism even in solitary prey. This

**Table 1 Average detectability scores**

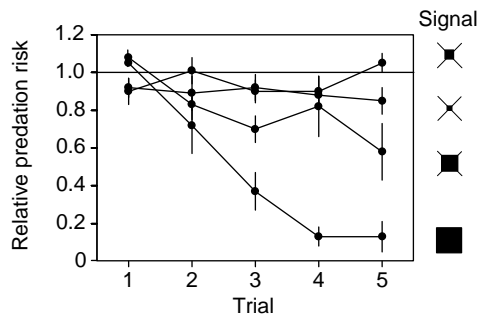
Signal type*	Visibility experiment			Learning experiment			Test	
	$\bar{x}$	s.e.m.	<i>n</i>	$\bar{x}$	s.e.m.	<i>n</i>	<i>U</i>	<i>P</i>
⊗	1.16	0.15	12	1.14	0.15	8	44	0.792
⊗	1.56	0.10	12	0.88	0.19	7	9	0.015
⊗	2.09	0.08	12	1.26	0.20	8	10	0.009
■	2.72	0.22	12	2.16	0.36	8	33	0.270

\* The four symbols represent a detectability continuum from the weakest signal type (first row) to the most conspicuous signal type (last row). Average detectability scores reflect the visibility risk for the non-cryptic prey types compared with the cryptic type. Detectability scores for the visibility experiment are averages from two trials. Because unpalatability might interfere with the visibility risks, the detectability scores for the initial phase of the learning experiment are counted from the five first prey items taken on the first trial. We compared these two situations (Mann-Whitney *U*-tests, Bonferroni corrected *P*-values) for each symbol type. There seemed to be a slight reduction in visibility risks during the learning experiment which might result from neophobia.

hypothesis was modelled recently by Yachi and Higashi<sup>10</sup>.

Using the novel-world method<sup>13</sup>, where naive predators are presented with warning signals not found in their environment, we conducted three experiments to test the gradual-change hypothesis. We studied the visibility differences, selective advantages of learning and generalization of avoidance of different levels of conspicuousness by creating a detectability continuum of artificial prey types. We created one cryptic signal and four aposematic signals, which ranged from nearly cryptic to highly conspicuous in relation to their background (see Fig. 2 for signals). Wild great tits (*Parus major*) were used as predators. To test the visibility differences of the five signals, eight palatable solitary items of each signal type were presented simultaneously. Birds received all signal types before the test in order to avoid any neophobic reactions. There were clear visibility differences between the signal types ( $n = 12$ , Friedman  $\chi^2 = 25.13$ , d.f. = 4,  $P < 0.001$ ; Table 1), except for the cryptic type and the weakest aposematic signal type (after sequential Bonferroni corrections; Wilcoxon  $T = -0.82$ ,  $P = 0.500$ ). The most conspicuous signal type was observed by the birds nearly three times more often than the cryptic type, which highlights the increased detectability risk.

A central question in understanding the evolution of warning coloration is the capability of predators to distinguish unpalatable from palatable prey. To test this, we devised a learning experiment in which a new set of birds was divided into four treatments according to the aposematic signal type. Before the experiment, each bird was offered three cryptic prey items to mimic the situation that only the aposematic prey signal was unfamiliar. The birds were then tested on five consecutive days for their ability to learn to avoid their assigned signalling unpalatable prey from the palatable cryptic prey. The initial detectability differences were not as clear as in the visibility experiment where birds had experienced all prey types

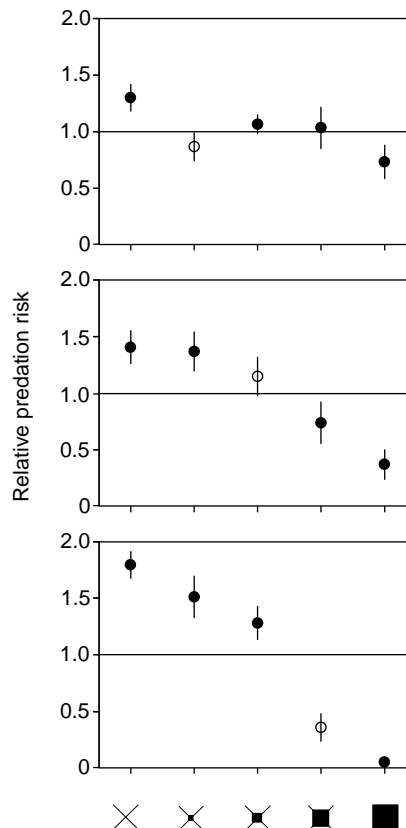


**Figure 1** The mean relative predation risk for all four aposematic signal types on five consecutive days in the learning experiment. The smallest square at the centre of cross is the weakest signal type, whereas the square that completely covers the cross is the most conspicuous signal type. The reference line indicates equal predation of cryptic and aposematic type. The bars represent standard errors of the mean (s.e.m.).

before testing (Table 1). The slight reduction in detectability differences might originate from neophobia<sup>15,16</sup>. There was also a significant interaction between the amount of unpalatable prey that birds ate in the five consecutive days in different signal treatments (repeated measure analysis of variance: learning  $\times$  signal,  $F(8, 36) = 2.74$ ,  $P = 0.018$ ). Although the least conspicuous aposematic signals do not suffer increased detectability risk, predators do not learn to avoid them (Fig. 1). The most detectable signals suffered higher predation at first, but they gained from avoidance learning. Learning was only significant when the signals were highly conspicuous (after Bonferroni corrections: second-most conspicuous signal, Friedman  $\chi^2 = 14.16$ , d.f. = 4,  $P = 0.027$ ; most conspicuous signal, Friedman  $\chi^2 = 19.81$ , d.f. = 4,  $P = 0.002$ ) and thus it seems that only the strongest signals can be avoided effectively.

One possible mechanism for the gradual-change pathway to evolve is through avoidance of even stronger signals by a process similar to a peak-shift effect<sup>17,18</sup>. Under this hypothesis, predators first learn to avoid a given signal and subsequently avoid exaggerated new signals even more. To test this idea, the birds from the learning experiment were presented with all four aposematic prey types simultaneously along with the cryptic palatable type from the cryptic background.

Throughout the learning experiment and training, the slight difference between the cryptic prey and the weakest signal was not sufficient for avoidance learning. The avoidance of more conspicuous signals than the weakest signal was not enhanced by any generalization effect and therefore the gradual-change route does not seem likely in the learning scenario (Friedman  $\chi^2 = 6.91$ ,



**Figure 2** The mean relative predation risk of different signals in the generalization experiment. The symbols along the abscissa are signal types. They represent a detectability continuum from the cryptic type (far left) through the four aposematic signal types from the weakest signal to the most conspicuous signal type (far right). The open circles indicate the signal that the birds had been trained to encounter as unpalatable. The bars represent s.e.m.

d.f. = 4,  $P = 0.141$ ; Fig. 2). Although the next signal (the third-strongest signal) was clearly detectable in the visibility experiment, birds had been unable to associate such prey as unpalatable during the learning experiment. After the additional training, birds shifted the avoidance of the learnt signal for the more conspicuous prey type (Friedman  $\chi^2 = 15.34$ , d.f. = 4,  $P = 0.004$ ). In the continuum, the first signal that birds learnt to avoid (the second-strongest signal) also had the advantage in the generalization experiment and this avoidance benefited the most conspicuous signal (Friedman  $\chi^2 = 37.5$ , d.f. = 4,  $P = 0.001$ ).

Weak signals that were not learnt did not suffer from increased predation, and thus any such mutations would not have been selected against. Thus, it is possible that stepwise mutations could occur by random drift until a level of conspicuousness is reached where predators can learn the signal. After this critical step the enhanced avoidance of predators might be generalized to more conspicuous signals without any initial detectability cost for the prey. □

**Methods**

The novel-world method allowed us to use wild great tits (*Parus major*) as naive predators. Birds were mist-netted (Jan–May 1997) near the Konnevesi research station and housed individually. Birds were trained to eat artificial prey items, pieces of almond placed between two 10 mm × 10 mm pieces of white paper that were glued together. In the experiments the prey items had printed symbols. All birds were familiarized to the testing situation by being allowed to feed on sunflower seeds and peanuts in the aviary before the experiment. A novel landscape was created in a 2.5 m × 5.2 m aviary, which was covered by white paper printed with 8 mm × 8 mm black crosses (580 crosses per m<sup>2</sup>). The cryptic prey items were made more difficult to detect by gluing fake prey items with cryptic symbols on the landscape (80 fakes per m<sup>2</sup>).

**Visibility experiment.** The continuum of signals from cryptic to conspicuous types was presented simultaneously on the novel landscape. All signals were palatable and eight items of each were used. Birds had previously received all of the different prey types (ten of each) in their housing cages to avoid neophobic reactions. The detectability scores were calculated from the first 20 prey items taken. The scores were calculated as a cumulative sum; the preferred prey type was given a value of 20 and the second-preferred prey type 19, and so on. The relations of the sums reflect the relative detectability risks of each prey type.

**Learning experiment.** Birds were tested individually on five consecutive days. Only one aposematic symbol was used over the course of the experiment for each bird. Cryptic prey items were palatable and aposematic prey items were made unpalatable by soaking almonds in a 40% solution of chloroquine. Prey items (24 aposematic and 24 cryptic) were scattered randomly on the landscape: the arena was divided into six equal-sized blocks, and four aposematic and four cryptic prey items were placed randomly in each block. Birds were allowed to eat 15 prey items in each daily experimental trial.

**Generalization experiment.** Avoidance towards the same unpalatable prey types that each bird had experienced in the learning experiment, was enhanced by offering further sets of cryptic and unpalatable prey (four replicates of five aposematic and five cryptic prey) simultaneously in their housing cages. In the experiment all aposematic prey types were presented simultaneously with cryptic items (eight items of each type). Birds were allowed to eat 15 prey items. After the experiments the birds were released back to sites where they were captured from.

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1. Wallace, A. R. *Trans. Entomol. Soc. Lond.* 3rd Ser. 3, lxxx–lxxxi (1867).
2. Schuler, W. & Roper, T. J. Responses to warning coloration in avian predators. *Adv. Study Behav.* 21, 111–146 (1992).
3. Gittleman, J. L. & Harvey, P. H. Why are distasteful prey not cryptic? *Nature* 286, 149–150 (1980).
4. Guilford, T. in *Insect Defences* (eds Evans, D. L. & Schmidt, J. O.) 23–61 (State Univ. New York Press, New York, 1990).
5. Guilford, T. & Dawkins, M. Receiver psychology and the evolution of animal signals. *Anim. Behav.* 41, 1–14 (1991).
6. Turner, J. R. G. & Mallet, J. L. B. Did forest islands drive the diversity of warningly coloured butterflies? Biotic drift and the shifting balance. *Phil. Trans. R. Soc. Lond. B* 351, 835–845 (1996).
7. Enderl, J. A. Frequency-dependent predation, crypsis and aposematic coloration. *Phil. Trans. R. Soc. Lond. B* 319, 505–523 (1988).
8. Enderl, J. A. in *Behavioural Ecology* (eds Krebs, J. R. & Davies, N. B.) 163–196 (Blackwell Scientific, Oxford, 1993).

9. Mallet, J. & Singer, M. C. Individual selection, kin selection, and the shifting balance in the evolution of warning colours: the evidence from butterflies. *Biol. J. Linn. Soc.* 32, 337–350 (1987).
10. Yachi, S. & Higashi, M. The evolution of warning signals. *Nature* 394, 882–884 (1998).
11. Leimar, O., Enquist, M. & Sillén-Tullberg, B. Evolutionary stability of aposematic coloration and prey unprofitability: a theoretical analysis. *Am. Nat.* 128, 469–490 (1986).
12. Fisher, R. A. *The Genetical Theory of Natural Selection* (Clarendon, Oxford, 1930).
13. Alatalo, R. V. & Mappes, J. Tracking the evolution of warning signals. *Nature* 382, 708–710 (1996).
14. Guilford, T. Is kin selection involved in the evolution of warning coloration? *Oikos* 45, 31–36 (1985).
15. Coppinger, R. P. The effect of experience and novelty on avian feeding behavior with reference to the evolution of warning coloration in butterflies I. Reactions of wild-caught adult blue jays to novel insects. *Behaviour* 35, 45–60 (1969).
16. Coppinger, R. P. The effect of experience and novelty on avian feeding behavior with reference to the evolution of warning coloration in butterflies II. Reactions of naive birds to novel insects. *Am. Nat.* 104, 323–335 (1970).
17. Hanson, H. M. Effects of discrimination training on stimulus generalization. *J. Exp. Psychol.* 58, 321–334 (1959).
18. Leimar, O. & Tuomi, J. Synergistic selection and graded traits. *Evol. Ecol.* 12, 59–71 (1998).

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## The K<sup>+</sup>/Cl<sup>-</sup> co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation

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GABA ( $\gamma$ -aminobutyric acid) is the main inhibitory transmitter in the adult brain, and it exerts its fast hyperpolarizing effect through activation of anion (predominantly Cl<sup>-</sup>)-permeant GABA<sub>A</sub> receptors<sup>1</sup>. However, during early neuronal development, GABA<sub>A</sub>-receptor-mediated responses are often depolarizing<sup>2,3</sup>, which may be a key factor in the control of several Ca<sup>2+</sup>-dependent developmental phenomena, including neuronal proliferation, migration and targeting<sup>4–6</sup>. To date, however, the molecular mechanism underlying this shift in neuronal electrophysiological phenotype is unknown. Here we show that, in pyramidal neurons of the rat hippocampus, the ontogenetic change in GABA<sub>A</sub>-mediated responses from depolarizing to hyperpolarizing is coupled to a developmental induction of the expression of the neuronal Cl<sup>-</sup>-extruding K<sup>+</sup>/Cl<sup>-</sup> co-transporter, KCC2 (ref. 7). Antisense oligonucleotide inhibition of KCC2 expression produces a marked positive shift in the reversal potential of GABA<sub>A</sub> responses in functionally mature hippocampal pyramidal neurons. These data support the conclusion that KCC2 is the main Cl<sup>-</sup> extruder to promote fast hyperpolarizing postsynaptic inhibition in the brain.

In the rat hippocampus, fast GABAergic transmission is depolarizing at birth, only becoming hyperpolarizing and strictly inhibitory by the end of the first postnatal week<sup>2,3</sup>. The molecular basis of this qualitative shift in GABA<sub>A</sub> action has not been established.

Northern blots of total messenger RNA extracted from rat hippocampus showed a pronounced developmental upregulation of the expression of KCC2 mRNA (Fig. 1a, b). At postnatal day 0 (P0; day of birth) KCC2 mRNA (5.5 kilobases (kb)) was barely detectable. However, a steep increase in expression was evident at P5, reaching a level at P9 that was essentially similar to that in the