do support spiking — these are clearly located within the axon terminals themselves [15,16]. Critically, the axon segment that Puthussery *et al.* found to be decorated with sodium channels in DB3/4 cells is not in the terminals themselves, but roughly half way between dendrites and axon terminals, thereby possibly allowing a more 'balanced' weighting of the axonal and dendritic synaptic input sites. However, a hypothetical strong inhibitory input near the active axon segment may permit direct gating control over spike generation.

If such a mechanism indeed exists, it would present one exciting possibility to explain 'mode switching' [9,13,15] — the idea that spiking in bipolar cells can be switched on and off depending on the system's current demands or recent input history. Notably, once initiated, a spike propagating from the active axon segment throughout the remainder of the cell could contribute to synchronizing the activity across all synaptic boutons, thereby counteracting potential bouton-specific independent signaling [17] in spiking bipolar cells. Clearly, more work is needed to reach a comprehensive understanding of active processes that occur deep within the terminal systems of different types of bipolar cells.

But Now for Something Completely Different

The specific subcellular localization of active conductances in bipolar cells also bears a more practical, that is, experimental, consideration. Puthussery *et al.* [1] note that it was notoriously difficult to reliably 'clamp' the axonal membrane potential when having the recording electrode located on the electrotonically distant soma. Accordingly, they interpreted runaway potentials that they occasionally observed as the result of an incomplete voltage clamp. And indeed, using biophysically realistic modeling they could directly recreate the experimentally observed effect when locating sodium channels to the said axonal segment. With retinal research homing in increasingly on the intricate processing strategies enabled by the interactions of bipolar cells, amacrine cells and ganglion cells, electrical recordings from the bipolar cell soma can clearly only scratch the surface. Current experimental strategies to complement classical electrophysiological approaches with optical imaging of calcium [18], glutamate release [19] and soon perhaps even voltage [20], will most certainly lead to exciting discoveries about the origin of parallel channels emerging from the retina.

References

1. Puthussery, T., Venkataramani, S., Gayet-Primo, J., Smith, R.G., and Taylor, W.R. (2013). NaV1.1 channels in axon initial segments of bipolar cells augment input to magnocellular Visual Pathways in the primate retina. J. Neurosci. *33*, 16045–16059.
2. Gollisch, T., and Meister, M. (2009). Eye smarter than scientists believed : neural computations in circuits of the retina. Neuron *65*, 150–164.
3. Polyak, S.L. (1941). The Retina (Chicago Univ. Chicago Press).
4. Shapley, R., and Hugh Perry, V. (1986). Cat and monkey retinal ganglion cells and their visual functional roles. Trends Neurosci. *9*, 229–235.
5. Wässle, H. (2004). Parallel processing in the mammalian retina. Nat. Rev. Neurosci. *5*, 747–757.
6. Awatramani, G.B., and Slaughter, M.M. (2000). Origin of transient and sustained responses in ganglion cells of the retina. J. Neurosci. *20*, 7087–7095.
7. Cui, J., and Pan, Z.-H. (2008). Two types of cone bipolar cells express voltage-gated Na+ channels in the rat retina. Vis. Neurosci. *25*, 635–645.
8. Zenisek, D., Henry, D., Studholme, K., Yazulla, S., and Matthews, G. (2001). Voltage-dependent sodium channels are expressed in nonspiking retinal bipolar neurons. J. Neurosci. *21*, 4543–4550.
9. Baden, T., Esposti, F., Nikolaev, A., and Lagnado, L. (2011). Spikes in retinal bipolar cells phase-lock to visual stimuli with millisecond precision. Curr. Biol. *21*, 1859–1869.
10. Baden, T., Euler, T., Weckström, M., and Lagnado, L. (2013). Spikes and ribbon synapses in early vision. Trends Neurosci. *36*, 1–9.
11. Ichinose, T., Shields, C.R., and Lukasiewicz, P.D. (2005). Sodium channels in transient retinal bipolar cells enhance visual responses in ganglion cells. J. Neurosci. *25*, 1856–1865.
12. Pan, Z.H., and Hu, H.J. (2000). Voltage-dependent Na(+) currents in mammalian retinal cone bipolar cells. J. Neurophysiol. *84*, 2564–2571.
13. Saszik, S., and DeVries, S.H. (2012). A mammalian retinal bipolar cell uses both graded changes in membrane voltage and all-or-nothing Na+ spikes to encode light. J. Neurosci. *32*, 297–307.
14. DeVries, S.H. (2000). Bipolar cells use kainate and AMPA receptors to filter visual information into separate channels. Neuron *28*, 847–856.
15. Burrone, J., and Lagnado, L. (1997). Electrical resonance and Ca^{2+} influx in the synaptic terminal of depolarizing bipolar cells from the goldfish retina. J. Physiol. *505*, 571–584.
16. Protti, D. a, and Llano, I. (1998). Calcium currents and calcium signaling in rod bipolar cells of rat retinal slices. J. Neurosci. *18*, 3715–3724.
17. Asari, H., and Meister, M. (2012). Divergence of visual channels in the inner retina. Nat. Neurosci *15*, 1581–1589.
18. Baden, T., Berens, P., Bethge, M., and Euler, T. (2012). Spikes in mammalian bipolar cells support temporal layering of the inner retina. Curr. Biol. CB, 1–5.
19. Borghuis, B.G., Marvin, J.S., Looger, L.L., and Demb, J.B. (2013). Two-photon imaging of nonlinear glutamate release dynamics at bipolar cell synapses in the mouse retina. J. Neurosci *33*, 10972–10985.
20. Jin, L., Han, Z., Platisa, J., Wooltorton, J.R.A., Cohen, L.B., and Pieribone, V.A. (2012). Single action potentials and subthreshold electrical events imaged in neurons with a fluorescent protein voltage probe. Neuron *75*, 779–785.

Centre for Integrative Neuroscience (CIN), Bernstein Centre for Computational Neuroscience (BCCN), Institute for Ophthalmic Research, University of Tübingen, Otfried-Müller Strasse 25, 72076, Tübingen, Germany.
E-mail: Thomas.Baden@uni-tuebingen.de, Thomas.Euler@cin.uni-tuebingen.de

http://dx.doi.org/10.1016/j.cub.2013.10.066

Conservation: Forest Fragments, Facts, and Fallacies

Most of the world's remaining habitats are split into small fragments that lose species quickly. Knowledge of this fact can guide practical actions to prevent extinctions.

Stuart L. Pimm[1,*] and Thomas Brooks[2]

Flights from Miami to Rio de Janeiro across the Amazon or from Detroit to Shanghai across Canada's and Russia's northern wilderness are exceptional journeys. They are exceptional because most plane trips pass over human-dominated landscapes. Not merely have we destroyed natural habitats worldwide, but what remains are mostly fragments. Our world is in tatters. Habitat fragments cover millions of square kilometres of Earth's surface. The fate of biodiversity depends on whether species will survive in them. Fragments

of forests are particularly important, as forests hold most species. A recent paper [1] extends the confirmation of earlier studies that small forest fragments lose many species and lose them quickly. Forests may remain, but empty of the wildlife they once housed. Ironically, the paper's implications support numerous other recent analyses in rejecting a speculation by one of its own authors [2] that previous studies exaggerated the likely species losses following habitat loss; to the contrary, they can be very much worse.

Species are geographically concentrated. Two-thirds of all plant species live exclusively within 17% of the land surface — an area that includes the northern Andes, the Caribbean, southern Africa, Madagascar and the islands of southeast Asia. A further 14% of species live in this same area plus other regions [3]. This same area houses 88% of bird, 82% of amphibian and 73% of mammal species [3–5]. Most unfortunately, these areas also suffer disproportionate damage from human activities, creating what Myers *et al.* [6] call 'biodiversity hotspots'. Thus, the coastal forests of Brazil, for instance, contain the largest concentrations of threatened bird species in the Americas (Figure 1) because this region retains only about 7% of its original forest and has an exceptional number of species endemic to it [3–5]. As satellite images show, the remaining forest is severely fragmented.

How well do species survive in these fragmented areas rich in endemic species — and what can conservation do to improve their chances? To answer the first question we need a seemingly impossible experiment. We should count species in areas before deforestation then follow their fate over the following decades in the newly formed forest fragments. Not surprisingly, very few studies can do this. In 1978, Thomas Lovejoy established with Brazilian colleagues the longest running fragment experiment in the Amazon rainforest north of Manaus [7]. Deliberately experimental, it created patches of different sizes (1, 10, or 100 hectares). The researchers counted the species found within them from before deforestation isolated them and then recounted in the following decades. By far the best data were obtained for birds [7]. The small fragments lost most of their species, and did so within a few years. Large fragments lost fewer species, over decades.

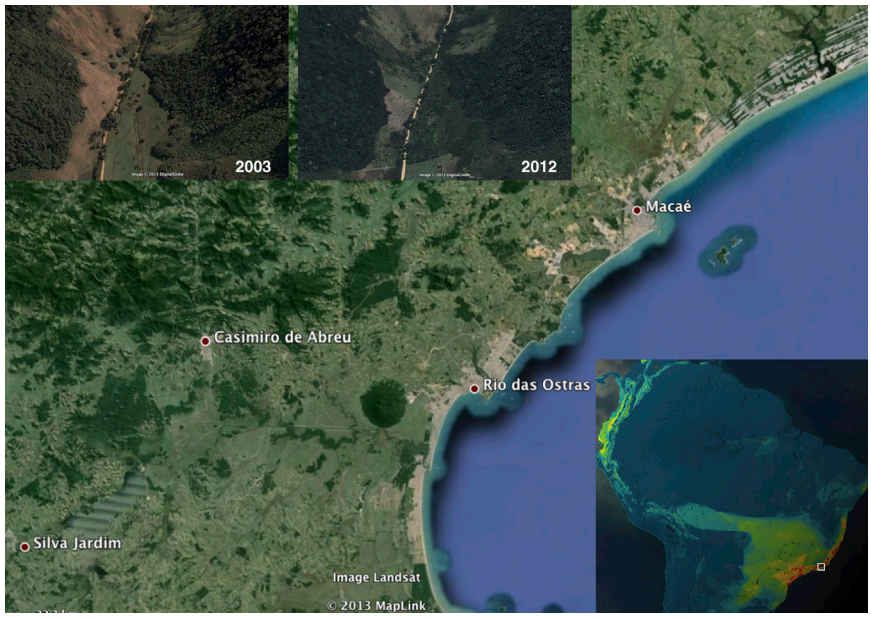

Figure 1. Fragmented landscapes sometimes hold the greatest concentrations of species at risk of extinction.

What habitats remain in biodiversity hotspots are often in small fragments, as in the coastal forests of Brazil. This region has more threatened species of birds than anywhere else in the Americas (bottom inset: blue, no threatened species; red, 23 species). As a consequence, the isolated Reserva Biológica União was expected to lose many species. (The reserve is isolated by a north to south road, just northeast of the word 'Ambreu' on the map.) The Brazilian NGO Associação Mico-Leão Dourado has now restored forest, providing habitat connections for the threatened golden lion tamarin and other species (top insets). (Satellite images courtesy of Google Inc. All rights reserved © 2013 MapLink © 2013 DigitalGlobe; species map courtesy of Clinton Jenkins and Felix Pharand-Deschenes of Globaia http://www.globaia.org.)

Other studies have combined historical forest cover and biodiversity data with modern surveys, for example, addressing how quickly bird species succumbed in rainforest patches around Kakamega, Kenya, after fragmentation [8]. Another type of fragmentation experiment is provided fortuitously by large dams creating islands of habitat as the land floods. The damming of the Xinanjiang River, China, in 1959, for instance, flooded 580 km^2, and formed 1078 islands with areas >0.25 ha [9]. Small islands held many fewer species of lizards than larger ones, when Wang *et al.* [9] surveyed the islands in 2007. Similarly, the flooding of the 4,300 km^2 Lago Guri, in Venezuela allowed Terborgh *et al.* [10] to document not only the loss of species from the islands created, but also the consequences of species loss throughout the changed food webs.

The new study of Gibson *et al.* [1] also focussed on fragmentation in a flooded area, namely Chiew Larn Reservoir in southern Thailand, which was flooded in 1986–1987 creating over 100 islands. The authors selected 16 islands in the reservoir ranging from 0.3 to 56.3 ha and surveyed small mammal communities five to seven years and 25 to 26 years after isolation. Two protected areas that form part of the largest (>3,500 km^2) contiguous forest area in southern Thailand surround the reservoir. In the first survey, islands larger than 10 ha had between seven and twelve species of small mammal, but this dropped to one to five species by the second survey. A further four similar islands surveyed in the second period had only one or two small mammal species on them. Smaller islands had two to three species in the first survey and only one to two in the second. With few exceptions, the only surviving species was the Malaysian field rat, *Rattus tiomanicus*, a human

commensal species. The results of this study [1] are similar to those obtained for birds in Manaus and Kakamega with regard to the rates at which species are lost. They are also comparable to a wealth of observations on habitat fragments that show how few species survive but where the time of isolation is unknown.

Despite this consistency of results from different experimental approaches, different places in the world, and now, thanks to Gibson *et al.* [1], different taxonomic groups, studies of fragmentation generate controversy. Some authors contest how severely and how fast habitat loss and fragmentation affect extinction. Some journalists have considered large-scale extinctions from deforestation as 'doomsday myths', noting how few bird species were lost after forest clearing in eastern North America, and that none were lost from coastal Brazil. A recent special issue of *The Economist* [11] on biodiversity repeated these assertions. Both are false.

Media misinterpretation may not surprise, but the source of the claim that few extinctions follow habitat fragmentation does: Fangliang He, a co-author of the Chiew Larn Reservoir study, and Stephen Hubbell published a study [2] titled 'Species-area relationships always overestimate extinction rates from habitat loss'. The media quoted them saying: "key measures of species loss in the 2005 UN Millennium Ecosystem Assessment and the 2007 Intergovernmental Panel on Climate Change (IPCC) report are based on fundamentally flawed methods that exaggerate the threat of extinction […] The International Union for the Conservation of Nature (IUCN) "Red List" of endangered species — likewise a benchmark for policy makers — is now also subject to review"[12]. These statements were quite remarkable, challenging the consensus opinions of authoritative science-policy interfaces involving thousands of scientists. The comment about the Red List is particularly egregious, because the Red List process assesses extinction risks species-by-species and rather than by modelling the aggregate effects of habitat fragmentation on all species in a region. As an on-going assessment process the Red List is, in any case, subject to constant review [4].

Turning to the methods considered 'fundamentally flawed' by He and Hubbell [2], these invoke the theory of island biogeography that notices that the number of species, S, scales with island area, A, according to $S = cA^z$, where c and z are constants. The latter is about 1/4, so an area one half the size of another will contain $(0.5)^{(1/4)} = 84\%$ of the larger area's species. The extension to habitat fragments is that one supposes that "habitat islands" behave similarly. It allows our scaling from understanding the effects of local fragmentation to global extinctions. For example, after the shrinking of North America's eastern forests to one half their original size from European colonisation to the low point in the 1870s, one should lose 16% (100% − 84%) of the region's species. Indeed, this figure is very close to the right answer for birds [13]. Four bird species went extinct [13]. It was so few, because most of the region's species occur well outside it and would have survived somewhere even if all the eastern forest had been cut. Similarly, the deforestation of the Brazilian coastal forests accurately predicts the number of the region's species threatened with global extinction [14]. This and many other empirical confirmations [15,16] did not apparently convince He and Hubbell to reassess their claims.

A subsequent flurry of publications confirms that it was indeed He's and Hubbell's calculations that were flawed. Their model considers only instantaneous extinctions, not the many more occurring over time as populations dwindle below a threshold where they persist [8,14,15,17]. The analytical basis for their claims represents a highly unlikely special case — that of deforestation starting from the centre of a forest and proceeding outwards [18]. The empirical basis for their work was based on inconsistent spatial sampling [19]. Moreover, the calculations of species losses have an obvious error, in that they assumed that the remaining "forest island" was near enough in one piece [20]. Were remaining fragments to be in tiny pieces, then even considerable remaining habitat, in total, might nonetheless, lose all its species — more, not less, than expected [20]. Nothing illustrates this better than the study of mammals in Chiew Larn Reservoir [1].

In summary, an ever-increasing body of work, both theoretical and empirical, shows that habitat fragmentation drives extinction. So what measure can we take against this? One obvious answer is to reconnect isolated fragments. Such an example involves the isolated "forest island" that was Reserva Biológica União (Figure 1). Note the 'was'. The Brazilian NGO Associação Mico-Leão Dourado bought the intervening cattle pasture. Five years later, forest has grown back and is readily visible on satellite images. Importantly, the endangered golden lion tamarin (*Leontopithecus rosalia*), once imprisoned in its forest island, are now able to connect with populations elsewhere. With concerted action, habitat islands need not be isolated forever and we can stop the near total loss of species from small fragments.

References

1. Gibson, L., Lynam, A.J., Bradshaw, C.J., He, F., Bickford, D.P., Woodruff, D.S., Bumrungsri, S., and Laurance, W.F. (2013). Near-complete extinction of native small mammal fauna 25 years after forest fragmentation. Science *341*, 1508–1510.
2. He, F., and Hubbell, S.P. (2011). Species-area relationships always overestimate extinction rates from habitat loss. Nature *473*, 368–371.
3. Joppa, L., Visconti, P., Jenkins, C., and Pimm, S. (2013). Achieving the convention on biological diversity's goals for plant conservation. Science *341*, 1100–1103.
4. BirdLife International, NatureServe (2011). *Bird species distribution maps of the world* (BirdLife International, Cambridge, UK and NatureServe, Arlington, USA).
5. IUCN (2010). IUCN Red List of Threatened Species. Version 2010.4.
6. Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A., and Kent, J. (2000). Biodiversity hotspots for conservation priorities. Nature *403*, 853–858.
7. Ferraz, G., Russell, G.J., Stouffer, P.C., Bierregaard, R.O., Pimm, S.L., and Lovejoy, T.E. (2003). Rates of species loss from Amazonian forest fragments. Proc. Natl. Acad. Sci. USA *100*, 14069–14073.
8. Brooks, T.M., Pimm, S.L., and Oyugi, J.O. (1999). Time lag between deforestation and bird extinction in tropical forest fragments. Conserv. Biol. *13*, 1140–1150.
9. Wang, Y., Zhang, J., Feeley, K., Jiang, P., and Ding, P. (2009). Life-history traits associated with fragmentation vulnerability of lizards in the Thousand Island Lake, China. Anim. Conserv. *12*, 329–337.
10. Terborgh, J., Lopez, L., Nunez, P., Rao, M., Shahabuddin, G., Orihuela, G., Riveros, M., Ascanio, R., Adler, G.H., and Lambert, T.D. (2001). Ecological meltdown in predator-free forest fragments. Science *294*, 1923–1926.
11. Budiansky, S. (1993). The Doomsday Myths. U.S. News and World Report, 81–91.
12. The Daily Star (2011). Extinction rate overestimated. http://archive.thedailystar.net/newDesign/news-details.php?nid=186495.
13. Pimm, S.L., and Askins, R.A. (1995). Forest losses predict bird extinctions in eastern North America. Proc. Natl. Acad. Sci. USA *92*, 9343–9347.
14. Brooks, T., and Balmford, A. (1996). Atlantic forest extinctions. Nature *380*, 115.

15. Brooks, T., and Pimm, S. (2011). Extinctions: consider all species. Nature *474*, 284.
16. Fattorini, S., and Borges, P.A. (2012). Species-area relationships underestimate extinction rates. Acta Oecologica *40*, 27–30.
17. Tanentzap, A.J., Walker, S., Theo Stephens, R., and Lee, W.G. (2012). A framework for predicting species extinction by linking population dynamics with habitat loss. Conserv. Letters *5*, 149–156.
18. Pereira, H.M., Borda-de-Água, L., and Martins, I.S. (2012). Geometry and scale in species-area relationships. Nature *482*, E3–E4.
19. Axelsen, J.B., Roll, U., Stone, L., and Solow, A. (2013). Species-area relationships always overestimate extinction rates from habitat loss: comment. Ecology *94*, 761–763.
20. Hanski, I., Zurita, G.A., Bellocq, M.I., and Rybicki, J. (2013). Species–fragmented area relationship. Proc. Natl. Acad. Sci. USA *110*, 12715–12720.

[1]Nicholas School of the Environment, Duke University, Box 90328, Durham, NC 27708, USA. [2]International Union for Conservation of Nature, 28 rue Mauverney, CH-1196 Gland, Switzerland.
*E-mail: stuartpimm@me.com

http://dx.doi.org/10.1016/j.cub.2013.10.024

Development: Getting into the Groove, or Evolving off the Rails?

Canalization, the robust buffering against fluctuations, is often regarded as an essential feature of development. A new study identifies a genetic circuit dedicated to canalization in *Drosophila melanogaster* and shows striking variability in its use in different fly species.

Kristen A. Panfilio and Siegfried Roth

The term canalization was first used by the embryologist and theoretician C.H. Waddington [1] to explain the observation that development nearly always generates the same, successful phenotypic outcome despite potential external disturbances or inherent noise. Waddington depicted this concept graphically as a ball rolling through a 'developmental landscape', where the ball's path represents the developmental trajectory of a single organism against the landscape of possible paths [2]. As the ball moves through the landscape, the path becomes increasingly determined as the landscape becomes increasingly steep, with clear valleys through which the ball can roll. These valleys are thus the 'channels' into which canalized development is directed. What aspects of development contribute to the steepening of the valley's slopes, and when? In a recent paper in *Current Biology* [3], the Ferguson lab at the University of Chicago has identified multiple, successive mechanisms for achieving canalization in the fruit fly *Drosophila melanogaster*, significantly strengthening the body of evidence for this phenomenon in a nuanced way, and uncovering new functional requirements for some known developmental genes.

The authors focus on the role of the BMP signaling pathway in specifying the dorsalmost tissue fate in the *Drosophila* embryo, the amnioserosa (Figure 1). Amnioserosa formation depends on high levels of BMP signaling in a narrow domain straddling the dorsal midline of the embryo. How this peak of BMP signaling is generated is still not fully understood. At least two processes are involved. The initiating mechanism is a reaction-diffusion system. The broadly transcribed BMP ligand (Decapentaplegic) becomes spatially restricted as an active signaling molecule through the localized transcription and extracellular diffusion of an inhibitor (Short gastrulation) that is itself cleaved and inactivated by a broadly distributed protease (Tolloid) [4]. In theory, as shown by computer simulations, this reaction-diffusion system can produce a refined BMP signaling peak with remarkable precision, given the right values for the rates of diffusion, decay and complex formation [5]. In reality, however, reaction and diffusion alone lead to only a slight enhancement of BMP signaling at the dorsal side [3,6]. A transcriptional feedback mechanism is additionally required to enhance receptor sensitivity, as the Ferguson lab had shown earlier by an ingenious set of experiments [6], giving rise to new modeling approaches [7–9]. The experiments described in their new paper [3] were designed to elucidate the nature of this transcriptional feedback. The authors admit that they still have not found all components, and how receptor sensitivity is enhanced at the biochemical level remains elusive. Even so, the new data provide an interesting facet of the system by identifying a feedback circuit that is not required for pattern formation *per se*, but for reducing noise in the patterning process, *i.e.*, for canalization.

By searching for genes expressed in the dorsal region, where BMP signaling refinement takes place, the authors focused on two genes, Eiger (Egr) and Crossveinless 2 (Cv-2), and showed that they are involved in regulating BMP signaling levels, quantified at the level of the BMP transducer pMad. The transmembrane Tumor Necrosis Factor-α homologue Egr acts cell autonomously to promote BMP signaling via the JNK pathway. At the same time, the extracellular, diffusible BMP-binding protein Cv-2 primarily acts as a BMP antagonist. A local, non-diffusible activator coupled with a diffusible inhibitor might provide the ideal prerequisite for a patterning system refining BMP signaling [10]. Indeed, BMP signaling is affected in *egr* and *cv-2* single mutants, with halving or doubling of pMad signal intensity, respectively, and with alterations in signal domain width. However, surprisingly the *egr cv-2* double mutant shows normalized signaling levels that are comparable to wild type. So, what is the raison d'être of this circuitry? Only statistical evaluation reveals the answer. In wild type and the single mutants, BMP levels are changed in a reproducible way with little variation across individual embryos. Also, the resulting number of amnioserosal cells increases or decreases in a corresponding manner. In contrast, this reproducibility is lost in the double mutant. BMP signaling levels and the number of amnioserosa cells become highly variable. The process is 'de-canalized'. In further evidence for de-canalization, the authors show that this genetic background sensitizes the embryo to a downstream BMP pathway mutant that normally has no phenotypic effect, exemplifying the