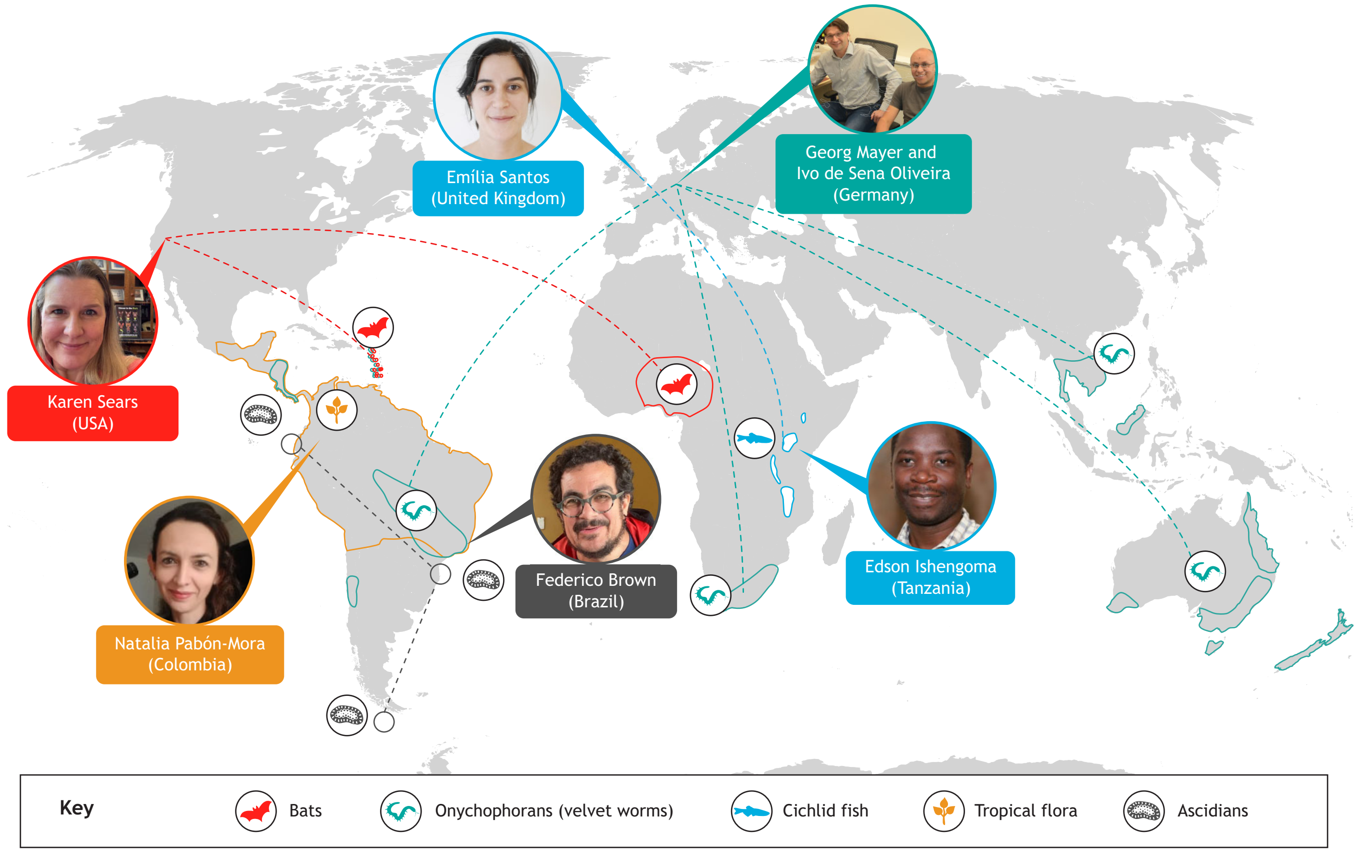
© 2024. Published by The Company of Biologists Ltd | Development (2024) 151, dev203084. doi:10.1242/dev.203084

Uncovering developmental diversity in the field

Federico D. Brown[1,2,3,*,‡‡], Edson Ishengoma[4,‡,‡‡], Georg Mayer[5,§,‡‡], Natalia Pabón-Mora[6,¶,‡‡], M. Emília Santos[7,‡,‡‡], Karen E. Sears[8,,‡‡] and Ivo de Sena Oliveira[5,§,‡‡]**



Contributors to this article, their geographical location and fieldwork destinations for sampling organisms of interest. In alphabetical order by last name: Federico D. Brown (Brazil), Edson Ishengoma (Tanzania) and Emília Santos (UK), Georg Mayer and Ivo de Sena Oliveira (Germany), Natalia Pabón-Mora (Colombia) and Karen Sears (USA).

ABSTRACT

Many developmental biologists seldom leave the lab for research, relying instead on establishing colonies of traditional and emerging model systems. However, to fully understand the mechanisms and principles of development and evolution, including the role of ecology and the environment, it is important to study a diverse range of organisms in context. In this Perspective, we hear from five research teams from around the world about the importance and challenges of going into the field to study their organisms of interest. We also invite you to share your own fieldwork stories on the Node.

[1]Departamento de Zoologia - Instituto Biociências, Universidade de São Paulo, 05508-090 São Paulo, Brazil. [2]Prometeo-Senescyt Program, Escuela Superior Politécnica del Litoral, ESPOL, P.O. Box 09-01-5863, Guayaquil 090902, Ecuador. [3]Centro de Biologia Marinha (CEBIMar), Universidade de São Paulo, 11612-109, São Sebastião, Brazil. [4]Department of Biological Sciences, Mkwawa University College of Education, University of Dar es Salaam, Dar es Salaam, Tanzania. [5]Department of Zoology, University of Kassel, Heinrich-Plett-Str. 40, 34132 Kassel, Germany. [6]Universidad de Antioquia, Instituto de Biología, Grupo Evo-Devo en Plantas, Medellín 050010, Colombia. [7]Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, UK. [8]Department of Ecology and Evolutionary Biology and Department of Molecular, Cellular, and Developmental Biology, University of California at Los Angeles, Los Angeles 90095, USA.
*This author contributed exclusively to 'Evolution of developmental systems in marine invertebrate life histories'. ‡These authors contributed exclusively to 'Developmental diversity through the lens of cichlid evolution'. §These authors contributed exclusively to 'Developmental diversity of velvet worms'. ¶This author contributed exclusively to 'Exploring the developmental biology of rich tropical flora'. **This author contributed exclusively to 'Beyond the lab: mining the natural diversity of mammals'.

‡‡Authors for correspondence (fdbrown@usp.br; edson.ishengoma@udsm.ac.tz; georg.mayer@uni-kassel.de; lucia.pabon@udea.edu.co; es754@cam.ac.uk; ksears@ucla.edu; ivo.oliveira@uni-kassel.de)

F.D.B., 0000-0001-9250-5011; E.I., 0000-0001-5012-8625; G.M., 0000-0003-0737-2440; N.P.-M., 0000-0003-3528-8078; M.E.S., 0000-0003-3158-7935; K.E.S., 0000-0001-9744-9602; I.d.S.O., 0000-0002-8340-7660

Evolution of developmental systems in marine invertebrate life histories

Federico D. Brown

The identification of animal homologies has troubled naturalists for centuries. Despite many difficulties in the assignment of structural homologies among vertebrates, marine invertebrates present a vastly more difficult challenge because of their diverse morphologies, complex life histories and deep evolutionary divergence. As a grad student participating in the Comparative Invertebrate Embryology course at Friday Harbor Labs, I carefully drew embryos of more than 20 different invertebrate phyla, revealing an endless diversity of forms and developmental mechanisms. With this extreme phenotypic diversity (Fig. 1A,B), approaches that compare spatiotemporal gene expression and function to reveal structural homologies are insufficient to capture the complexity of homologies in invertebrate development. After the course, rather than seeing development as a stable system driven by rigid processes following only a handful of instructions, I began to understand developmental system evolution (Waddington, 1941) as an active and dynamic process of change during the evolution of form.

The study of invertebrates offers an exquisite opportunity to explore how development has influenced diverse body forms and animal organization through ~850 million years of evolution. To understand and capture the breadth of morphological complexity, a complete picture of evolving developmental systems becomes necessary. In my lab, we study colonial ascidians, which exhibit perpetual development and morphological transformation throughout their life cycle. In ascidians, cells are systematically undergoing developmental processes (i.e. differentiation and cell death) regulated by genetic factors and inductive signals of tissues/organs, and influenced by individual or colony-level modulation (Stolfi and Brown, 2015; Kassmer et al., 2019; Manni, 2023). Furthermore, because ascidian larvae are motile and adults are sessile, where and when metamorphosis occurs is highly influenced by substrate preference and other environmental effects. Thus, colonial ascidian development cannot be fully understood in isolation from environmental influences.

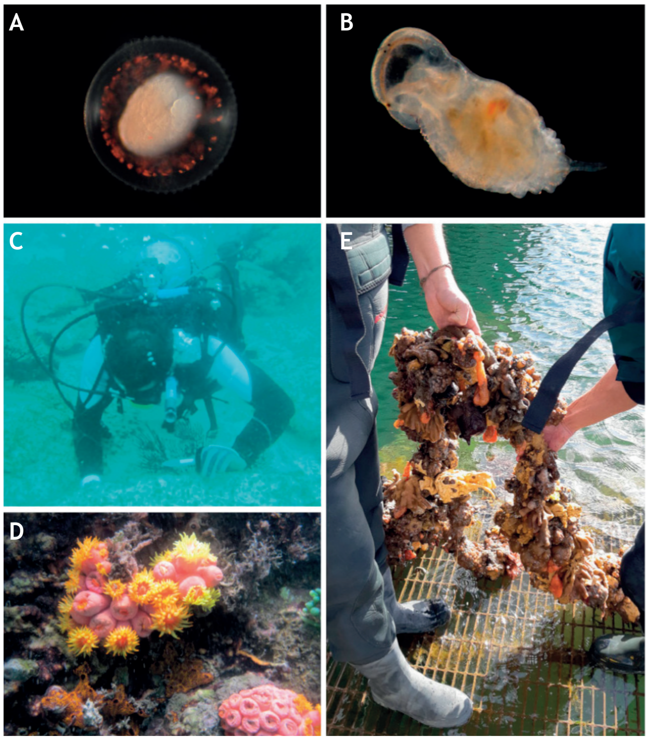


Fig. 1. Marine invertebrate fieldwork. (A,B) Diversity of organismal forms, as represented by a gastrula of the ascidian *Herdmania pallida* (A) and a metatrochophore larva of the polychaete *Chaetopterus variopedatus* (B); both were generated by *in vitro* fertilization in the Comparative Embryology of Marine Invertebrate (CEMI) course at the Center for Marine Biology of the University of São Paulo (CEBIMAR-USP). (C) Collecting minute colonial ascidians by scuba diving in the Eastern Tropical Pacific with colleague G. Agurto (CENAIM-ESPOL) at Bajo Cope, Ayangue, Santa Elena, Ecuador. (D,E) Comparison of marine invertebrate fouling communities in a subtropical region and a subantarctic region. (D) *Botrylloides niger* ascidian colonies competing for space with the invasive sun coral at a dock in the Ilhabela Yacht Club in the Channel of São Sebastião, Brazil. (E) Large colonial and solitary ascidians, including the colonial *Polyzoa opuntia* and the solitary *Paramolgula gigantea*, at the ferry dock in Porvenir, Tierra del Fuego, Chile. Images courtesy of A. Migotto (A,B; CEBIMAR-USP, Brazil) and J. I. Cañete (E; Universidad de Magallanes, Chile).

Colonial ascidian development cannot be fully understood in isolation from environmental influences

Field study approaches are advantageous to address developmental questions because patterns affecting development and evolution in nature (Fig. 1C) often go unseen in the laboratory. One biogeographic diversity pattern is the dominance (in species and abundance) of colonial ascidians in the tropics, where predation is highest (Fig. 1D,E). Our biogeographical and field studies have confirmed two peaks of colonial ascidian dominance near the tropics of Capricorn and Cancer, providing evidence for a major incidence of predation on colonial ascidians compared with solitary ones (Hiebert et al., 2019). These observations suggest that colonial ascidians – similar to other sessile marine animals – could have been selected to evolve high regenerative abilities, fast propagative growth and dormant forms to escape predation (Hiebert et al., 2019, 2022). A second pattern affecting development and evolution is polar gigantism. Low temperatures have been equivocally suggested to release oxygen-mediated body size constraints, leading to the evolution of gigantism in several marine taxa (reviewed by Woods and Moran, 2020). Solitary ascidians in the subantarctic Magellanic region (Chile) exhibit larger body size and relatively higher numbers of species, and colonial ascidians show relatively larger body and colony size than in the subtropical region of the São Sebastião Channel (Brazil) (Fig. 1D,E). Two other largely unexplored areas of research in tropical colonial ascidians include: understanding the evolutionary origin and developmental mechanisms of photosymbiosis in didemnid ascidians; and understanding the ecological role(s) of colour-morphs and their biogeographic distribution.

Altogether, global field studies capturing developmental diversity are necessary. On one hand, they reveal how the environmental affects developmental mechanisms and, on the other hand, they help us uncover how development responds to selection acting on the different levels of organismal organization. Biologists interested in the evolution of developmental systems face new challenges in understanding how developmental patterns of morphological diversity and adaptations affect reproduction, speciation and animal life histories.

Developmental diversity through the lens of cichlid evolution

M. Emília Santos and Edson Ishengoma

Fieldwork is a foundation of biological studies, offering unparalleled insights into the diversity of organisms. Its importance is exemplified by Darwin's and Wallace's expeditions studying organisms in their natural environments, leading to the ground-breaking theory of evolution by natural selection. One century later, the developmental mechanisms underpinning diversity, such as the conserved role of Hox genes in body plan development, started to be uncovered, contributing to the birth of evolutionary developmental biology (evo-devo) for understanding the evolution of developmental processes.

Traditionally, evo-devo has focused on a few laboratory model systems and often on major evolutionary transitions. Thus, studies on the developmental basis of organismal diversification and adaptation in their natural environments lagged behind. Recent technological advances have greatly expanded the genomic and developmental genetics toolkits, enabling the study of a broader range of organisms in their natural environments and giving rise to the field of eco-evo-devo, which integrates ecological, evolutionary and developmental perspectives.

Our eco-evo-devo research focuses on a diverse species: cichlid fishes (Santos et al., 2023). Cichlids outperform all other aquatic inhabitants of Africa in terms of diversification rates, particularly in the East African Great Lakes, where an extremely high density of fish show an immense diversity of forms and colours. An estimated 2000 cichlid species have emerged in East Africa in the past 5-7 million years (Salzburger, 2018). Despite their morphological diversity, these fish are genetically similar, providing a natural 'mutagenic screen' to map genetic and developmental mechanisms underlying diversity (Kocher, 2004; Malinsky et al., 2018). The well-documented biology and ecology of these fishes from fieldwork contributions of researchers and hobbyists provide crucial context for understanding genetic and developmental evolution.

Research in cichlids has highlighted the genetic and developmental basis of variation in adaptive traits, such as craniofacial shape and pigmentation patterns. Further studies on the modularity and integration of the cichlid craniofacial skeleton, and the genomic basis of convergent evolution in pigmentation patterns have shown how developmental mechanisms can facilitate or constrain adaptation and diversification (Conith and Albertson, 2021; Kratochwil et al., 2018). Comparative embryology among closely related cichlid species has revealed distinct developmental timelines and embryonic morphologies driven by local selective pressures and environmental conditions (Marconi et al., 2023; Powder et al., 2015).

Observing and characterising the environmental context is a major strength of fieldwork, allowing inference for how the environment drives phenotypic shifts and evolutionary change, and how it affects developmental processes within a generation through phenotypic plasticity. For example, cichlids develop different craniofacial and jaw structures depending on food availability (Gunter et al., 2013). This phenotypic plasticity allows rapid acclimatisation to environmental changes, a crucial factor in their evolutionary success.

Observing and characterising the environmental context is a major strength of fieldwork

An understanding of how developmental plasticity interacts with genetic variation during bouts of adaptation to novel environmental challenges is lacking. This question is the focus of our current collaboration, funded through the Cambridge-Africa Alborada program, where we will explore the repeated adaptation of cichlids to salinity gradients across important riverine ecosystems to understand mechanisms of evolutionary convergence and developmental plasticity in the wild. Such projects have the potential to contribute to both basic science and conservation. Understanding the adaptive capacity of key developmental pathways that enable species to adapt to changing environments can help devise conservation strategies.

Integrating field-based research with developmental biology is essential to understanding and preserving the natural world. It enriches our understanding of the interplay between genetics, development and the environment, revealing adaptive strategies and how developmental mechanisms influence diversification. Although invaluable, fieldwork must be conducted ethically, respecting and incorporating local knowledge and expertise, and developing equitable partnerships with local researchers.

Developmental diversity of velvet worms

Georg Mayer and Ivo de Sena Oliveira

Few scientists are familiar with onychophorans, also called velvet worms (Fig. 2A,B). Yet these creatures have always played an important role in discussions of animal development and evolution (Manton, 1949; Mayer et al., 2015). One major reason is their conserved morphology, which has changed little in hundreds of millions of years. Extant velvet worms still resemble fossil lobopodians – marine ancestors of onychophorans, tardigrades (water bears) and arthropods (e.g. crustaceans and insects). Owing to this legacy, onychophoran development may inform about the transformational changes that have led to the evolution and diversification of these three groups of panarthropods (Martin et al., 2017).

Onychophorans are predators that live in leaf litter and decaying logs in tropical and temperate forests of the southern hemisphere and around the equator, where they mostly feed on terrestrial arthropods, such as crickets and cockroaches. Prey is captured using

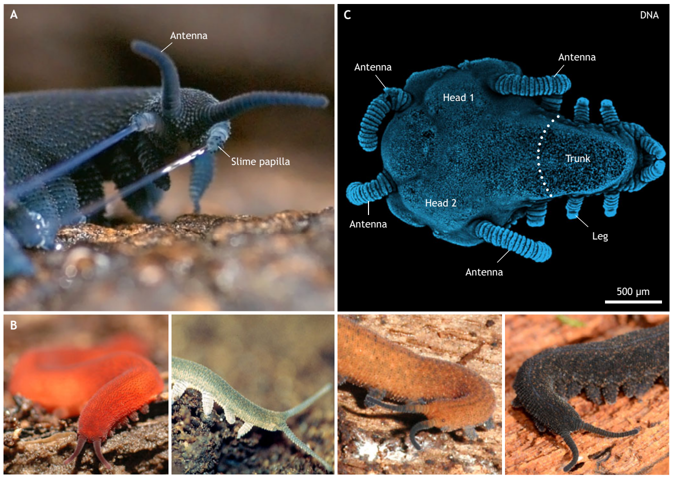


Fig. 2. Velvet worms and their developmental diversity. (A) A slime-ejecting specimen of *Euperipatoides rowelli* from Australia. (B) Photographs of onychophoran species, from left to right: *Epiperipatus biolleyi* (Costa Rica), *Peripatopsis capensis* (South Africa), *Occiperipatoides gilesii* (Australia), and *Ooperipatus oviparus* (Australia). (C) A double-headed *E. rowell* embryo with a truncated body from the hybrid zone in Tallaganda State Forest (Australia). Image was captured using a confocal microscope; DNA is labelled.

a sticky slime secretion, which is produced in specialized glands and ejected via a pair of modified limbs – the slime papillae (Fig. 2A). The skin of velvet worms is ultrahydrophobic and effectively repels water, dirt and even their own 'superglue'. Even though their collection does not require expensive equipment – gardening gloves, a screwdriver and a few jars – fieldtrips can be quite adventurous, especially to remote areas. In tropical forests, velvet worms are hard to find due to their cryptic lifestyle and low abundance. Furthermore, onychophorans are slow-moving animals that rarely fall into traps. Hence, successful onychophoran collection relies on well-trained eyes and luck.

The selection of the appropriate geographical region for fieldtrips is crucial, because onychophorans display a variety of developmental modes, each occurring in specific regions. For example, the placental live-bearing mode is exclusively found in the neotropics, whereas egg-laying representatives are restricted to Australia and New Zealand. A peculiar type of development involving huge trophic organs occurs in some South African, Chilean and Indonesian species. Which of these modes is ancestral to Onychophora is still under debate, but it seems clear that oviparity (deposition of eggs) has evolved at least twice (Mayer et al., 2015).

Covering the entire diversity of developmental modes is challenging because we must travel across the globe, find, collect and export the animals, inevitably facing country-related issues. It is, therefore, unsurprising that, in past decades, embryological research has focused on a few species, above all *Euperipatoides kanangrensis* and *E. rowelli* from Australia (Eriksson et al., 2003; Mayer et al., 2015; Janssen, 2017). Once collecting and export permits have been granted, these two species are easily obtainable in high numbers without affecting their natural populations.

Pioneering extensive fieldwork on these species, in particular *E. rowelli* (Fig. 2A), has revealed remarkable developmental abnormalities and low fertility in animals from specific areas of the forest (Sunnucks and Tait, 2001). The malformations include atypical numbers of segments, missing legs and jaws, and even double-headed embryos with truncated bodies (Fig. 2C). Fine-scale sampling and population genetics subsequently revealed that these developmental defects occur in 'hybrid zones', where the fitness of the animals is reduced, possibly because of the isolation of subpopulations of *E. rowelli* in glacial refuges during the Pleistocene about 2.6 million years ago (Bull and Sunnucks, 2014).

This example highlights the importance of combining field and laboratory work. Whereas observations in the field provide information about the biology of the species and the geology of the region, lab work exposes the underlying developmental and genetic mechanisms responsible for the adaptation of the species to its environment. This complementary approach allows us to relate the genotype to the phenotype, and broadens our view on the mutual relationship between the ecology, development and evolution of velvet worms.

> **Observations in the field provide information about the biology of the species and the geology of the region**

Exploring the developmental biology of rich tropical flora

Natalia Pabón-Mora

The American tropics contain some of the richest regions of biodiversity, with a range of niches where adaptation to extreme environments makes them natural laboratories (Marcellini et al., 2016). In Colombia, the three mountain ranges of the Andes form a mosaic of ecosystems, from mangroves, rainforests and savannas, as well as dry forests and hyper-humid cloud forests, to high and extensive Páramos and super-Páramos. These environments have shaped the diversification of native plant lineages with extraordinary morphological and co-evolutionary traits in need of thorough evolutionary and developmental studies.

> **These environments have shaped the diversification of native plant lineages with extraordinary morphological and co-evolutionary traits in need of thorough evolutionary and developmental studies**

For the past 12 years, we have developed four lines of research in neotropical plants. The first tackles the genetics of flowering, in which endogenous factors and environmental signals converge. We have studied flowering in neotropical orchids (Fig. 3A), generating a dataset of 14 reference transcriptomes in the search for flowering genes. Fieldwork helped stage the reproduction seasons of the populations and perform careful sampling for comparative transcriptomic analyses in selected species. We now know that, although the flowering genetic regulatory network has multiple duplications in orchids, only a few genes are transcriptionally active and seem important during flowering in the tropics. Moreover, this sampling has identified possible conserved flowering promoters and repressors (Madrigal et al., 2023).

Second, we have studied genes associated with floral organs in species that deviate from the standard floral organization, such as the Dutchman's pipe flowers (Aristolochiaceae). In the absence of true petals, they have attractive, kettle-shaped petal-like sepals with outstanding epidermal modifications such as papillae and trichomes (Fig. 3B) (Pabón-Mora et al., 2015, 2020; Pérez-Mesa et al., 2020; Muñoz-Gómez et al., 2021; Suárez-Baron et al., 2016, 2021, 2023). Fieldwork was instrumental in identifying pollinators and obtaining our living collections for developmental studies. Similarly, nasturtiums, belonging to an American family, the Tropaeolaceae (Martínez-Salazar et al., 2021, 2023), have a floral nectar spur that strongly shifts the floral symmetry from radial to bilateral (Fig. 3C).

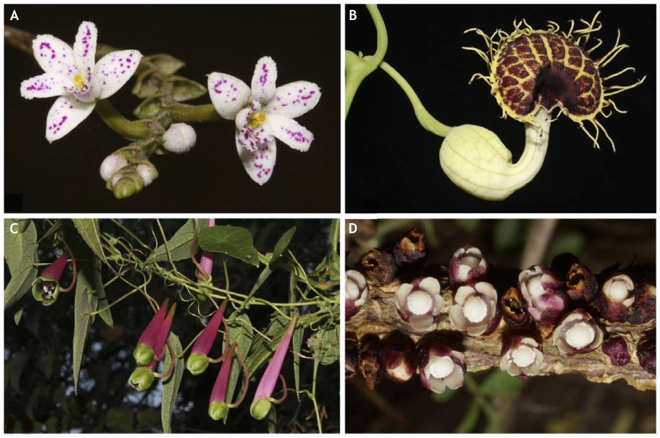


Fig. 3. Neotropical plant species. (A) The miniature tropical orchid *Epidendrum fimbriatum*. (B) A Dutchman's pipe flower, *Aristolochia fimbriata*, in anthesis. (C) Nasturtiums. Note the floral spur in *Tropaeolum longifolium*. (D) Flowers of *Pilostyles boyacensis*.

We have identified key genes for spur elaboration that likely trigger increased cell division rates in the overdeveloped side of the flower (Martínez-Salazar et al., 2021, 2023).

A third area is identifying anatomical and genetic variation during the carpel-to-fruit ontogenetic transition, focusing on the tomato and tobacco family (Solanaceae) (Ortíz-Ramirez et al., 2019; Hernández-Ciro and Pabón-Mora, 2020), the coffee family (Rubiaceae) (Salazar-Duque et al., 2021), mistletoes (Loranthaceae) and the páramo hail lineage (Chloranthaceae). Field observations have been instrumental in recording morphological shifts and seeing fruit dispersers in action, but also in pinpointing the most crucial time points in development for key transitions.

Finally, we have studied parasitic plant biology, with special attention to members of the Apodanthaceae, a lineage of holoparasitic species that have lost roots, stems and leaves. They live as small clusters of cells within other plants, forming only flowers and fruits at the expense of their host and emerge from the host to complete their life cycle (Fig. 3D) (González and Pabón-Mora, 2017). We have identified large reductions in their plastome (Arias-Agudelo et al., 2019) and crucial reductions in the genetic networks controlling their vegetative development (González et al., 2020). We are currently studying flowering mechanisms, as well as genes involved in flowering and floral organ identity in two species that grow in extreme environments: *Apodanthes caseariae*, which is primary parasite of Casearia trees (Salicaceae) in humid forests; and *Pilostyles boyacensis*, which is and exclusive parasite of *Dalea cuatrecasasii* (Fabaceae) in dry inter Andean thickets. As no holoparasitic taxa can even be germinated in the lab without a host plant, fieldwork is crucial in studying such intricate symbioses to record development and sample specimens *in situ*.

Beyond the lab: mining the natural diversity of mammals

Karen E. Sears

My research group seeks to harness the diversity of mammals to identify intrinsic processes driving change in organisms during their lives and in lineages over time. Although we can learn a lot about these topics from traditional lab mammals, the world beyond the lab contains thousands of mammalian species, each of which represents an evolutionary experiment. With this in mind, we have embarked on fieldwork on several continents to mine the information contained in these 'natural mutants'. For example, we routinely engage in collaborative fieldwork on dozens of bat species at sites in Central America and the Caribbean (e.g. Belize, Dominican Republic, Puerto Rico and Trinidad) and are now establishing sites in Africa (e.g. Cameroon and Nigeria). However, although there is almost no end to what we can learn from the study of wild mammals, fieldwork, and in particular fieldwork for the study of developmental biology, presents unique challenges and therefore requires a unique mindset.

As I have said on occasion, 'developmental biology is hard, developmental biology in mammals is harder, and developmental biology in non-model mammals is just ridiculous'. I say this with love – I love being in the field and I learn so much from seeing animals in their natural habitat – however, I also say this from experience. You have to get to the field, get funding, obtain permits and coordinate the movements and goals of a number of scientists. Once you get there, you need to find shelter and keep yourself and your crew fed and healthy. You need to obtain specimens. Weather can be uncooperative and mammals difficult to catch, and even when you do catch them, they might not be pregnant or be at a stage of pregnancy other than that you are interested. You have to process specimens in the field, which can be confounded by loss of electricity, equipment loss or breakage, contamination, fatigue, etc. You have to get your specimens back to your lab and/or do your assays in the field, both of which can present significant difficulties.

I say this with love – I love being in the field and I learn so much from seeing animals in their natural habitat

Personally, I have found that the best way to mitigate the challenges and uncertainties presented by fieldwork is by coupling loads of upfront planning with on-the-ground flexibility. One needs to be prepared for any situation and ready to change plans on the fly. As someone living with an anxiety condition, this is often my biggest challenge when engaging in fieldwork. I find that flexibility is facilitated by having multiple projects in the pipeline, each of which requires different field samples and/or developmental stages. That way, whatever tissues you collect, they will probably be useable. I also prioritize building collaborative and mutually beneficial working relationships with local scientists and organizations. Although beyond the scope of this brief perspective, fieldwork has a long history of exploitative collection. I have established a Summer Internship Program for Puerto Rican undergraduates and I am collaborating with the Fellowship Program of the Small Mammal Conservation Organization of West Africa, among other groups, to try to help develop in-country capacity for scientific research at our field sites and beyond. However, there is much more work to be done.

Acknowledgements
F.D.B. thanks A. Morillo for his comments on the first version of the text; G. Agurto, S. Tiozzo and J.I. Cañete for their help and support during the fieldwork described here; A. Migotto and A. Morandini for their contributions during the CEMI courses at CEBIMAR; and all the student participants in the course for their enthusiasm. F.D.B. thanks their professors E. del Pino, B. Swalla, T. De Tomaso, R. Strathmann, and G. von Dassow for introducing me to the diversity of developmental processes in marine invertebrates; and the Ilhabela Yacht Club (YCI), CEBIMAR and CENAIM staff for all the support. G.M. thanks P. Sunnucks, D. M. Rowell, N. N. Tait and P. M. Whitington for their invaluable support over the past two decades. N.P.-M. acknowledges all students in the Plant Evo Devo group, especially those that have accompanied her on fieldwork in isolated or hard to reach places in the Andes; and Favio Gonzalez (Universidad Nacional de Colombia) and Juan Fernando Alzate (Universidad de Antioquia) for their immense contributions during fieldwork and lab work. K.E.S. thanks all those with whom she has worked in the field.

Competing interests
The authors declare no competing or financial interests.

Funding
F.D.B. was funded by a Prometeo-Senescyt Research Award (Ecuador) and by the Fundação de Amparo à Pesquisa do Estado de São Paulo (projects 2015/50164-5, 2018/50017-0 and 2019/06927-5). E.I. and M.E.S. are supported by the Cambridge-Africa ALBORADA research fund. G.M. is funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation; MA 4147/12-1 and GRK 2749: 'Biological Clocks on Multiple Time Scales'). N.P-M.'s research on fruit development has been funded by a European Union Horizon 2020 research and innovation programme under a Marie Skłodowska–Curie grant (101007738 Evofruland). M.E.S. is supported by a Natural Environment Research Council IRF (NE/R01504X/1). K.E.S.'s fieldwork has been supported by grants from the National Science Foundation (2017803, 1854469, 1707519 and 1256423) and the National Institutes of Health (5R21AG078784), and by National Geographic. I.S.O. is supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq Brazil; 290029/2010-4).

Special Issue
This article is part of the Special Issue 'Uncovering developmental diversity', edited by Cassandra Extavour, Liam Dolan and Karen Sears. See related articles at https://journals.biologists.com/dev/issue/151/20.

References

Arias-Agudelo, L. M., González, F., Isaza, J. P., Alzate, J. F. and Pabón-Mora, N. (2019). Plastome reduction and gene content in New World Pilostyles (Apodanthaceae) unveils high similarities to African and Australian congeners. *Mol. Phylogenet. Evol.* **135**, 193-202. doi:10.1016/j.ympev.2019.03.014

Bull, J. K. and Sunnucks, P. (2014). Strong genetic structuring without assortative mating or reduced hybrid survival in an onychophoran in the Tallaganda State Forest region, Australia. *Biol. J. Linn. Soc.* **111**, 589-602. doi:10.1111/bij.12224

Conith, A. J. and Albertson, R. C. (2021). The cichlid oral and pharyngeal jaws are evolutionarily and genetically coupled. *Nat. Commun.* **12**, 5477. doi:10.1038/s41467-021-25755-5

Eriksson, B. J., Tait, N. N. and Budd, G. E. (2003). Head development in the onychophoran Euperipatoides kanangrensis. With particular reference to the central nervous system. *J. Morphol.* **255**, 1-23. doi:10.1002/jmor.10034

González, F. and Pabón-Mora, N. (2017). Floral development and morphoanatomy in the holoparasitic Pilostyles (Apodanthaceae, Cucurbitales) reveals chimeric half-staminate and half-carpellate flowers. *Int. J. Plant Sci.* **178**, 522-536. doi:10.1086/692505

González, A. D., Pabón-Mora, N., Alzate, J. F. and González, F. (2020). Meristem genes in the highly reduced endoparasitic Pilostyles boyacensis (Apodanthaceae). *Front. Ecol. Evol.* **8**, 209. doi:10.3389/fevo.2020.00209

Gunter, H. M., Fan, S., Xiong, F., Franchini, P., Fruciano, C. and Meyer, A. (2013). Shaping development through mechanical strain: the transcriptional basis of diet-induced phenotypic plasticity in a cichlid fish. *Mol. Ecol.* **22**, 4516-4531. doi:10.1111/mec.12417

Hernández-Ciro, N. and Pabón-Mora, N. (2020). Anatomical and genetic bases underlying convergent evolution of fleshy and dry dehiscent fruits in Cestrum and Brugmansia (Solanaceae). *Int. J. Dev. Biol.* **65**, 301-311. http://www.ijdb.ehu.es/web/paper.php?doi=200080np.

Hiebert, L. S., Vieira, E. A., Dias, G. M., Tiozzo, S. and Brown, F. D. (2019). Colonial ascidians strongly preyed upon, yet dominate the substrate in a subtropical fouling community. *Proc. Biol. Sci.* **286**, 20190396. doi:10.1098/rspb.2019.0396

Hiebert, L. S., Scelzo, M., Alié, A., De Tomaso, A. W., Brown, F. D. and Tiozzo, S. (2022). Comparing dormancy in two distantly related tunicates reveals morphological, molecular, and ecological convergences and repeated co-option. *Sci. Rep.* **12**, 12620. doi:10.1038/s41598-022-16656-8

Janssen, R. (2017). A molecular view of onychophoran segmentation. *Arthropod. Struct. Dev.* **46**, 341-353. doi:10.1016/j.asd.2016.10.004

Kassmer, S. H., Nourizadeh, S. and De Tomaso, A. W. (2019). Cellular and molecular mechanisms of regeneration in colonial and solitary Ascidians. *Dev. Biol.* **448**, 271-278. doi:10.1016/j.ydbio.2018.11.021

Kocher, T. D. (2004). Adaptive evolution and explosive speciation: the cichlid fish model. *Nat. Rev. Genet.* **5**, 288-298. doi:10.1038/nrg1316

Kratochwil, C. F., Liang, Y., Gerwin, J., Woltering, J. M., Urban, S., Henning, F., Machado-Schiaffino, G., Hulsey, C. D. and Meyer, A. (2018). Agouti-related peptide 2 facilitates the convergent evolution of stripe patterns across cichlid fish radiations. *Science* **362**, 457-460. doi:10.1126/science.aao6809

Madrigal, Y., Alzate, J. F. and Pabón-Mora, N. (2023). Evolution of flowering integrators in Orchidaceae. *Plant Reprod.* **37**, 85-109. doi:10.1007/s00497-023-00482-7

Malinsky, M., Svardal, H., Tyers, A. M., Miska, E. A., Genner, M. J., Turner, G. F. and Durbin, R. (2018). Whole-genome sequences of Malawi cichlids reveal multiple radiations interconnected by gene flow. *Nat. Ecol. Evol.* **2**, 1940-1955. doi:10.1038/s41559-018-0717-x

Manni, L. (2023). The colonial tunicate Botryllus schlosseri: a key species for evolutionary developmental studies. *Genesis* **61**, e23544. doi:10.1002/dvg.23544

Manton, S. M. (1949). Studies on the Onychophora VII. The early embryonic stages of Peripatopsis, and some general considerations concerning the morphology and phylogeny of the Arthropoda. *Philos. Trans. R. Soc. B, Biol. Sci.* **233**, 483-580.

Marcellini, S., González, F., Sarrazin, A., Pabón-Mora, N., Benitez, M., Piñeyro-Nelson, A., Rezende, G. L., Maldonado, E., Schenider, P., Bortoletto Grizante, M. et al. (2016). Evolutionary developmental biology (Evo-Devo) research in Latin America. *J. Exp. Zool. Part B.* **328**, 5-40. doi:10.1002/jez.b.22687

Marconi, A., Yang, C. Z., McKay, S. and Santos, M. E. (2023). Morphological and temporal variation in early embryogenesis contributes to species divergence in Malawi cichlid fishes. *Evol. Dev.* **25**, 170-193. doi:10.1111/ede.12429

Martin, C., Gross, V., Hering, L., Tepper, B., Jahn, H., Oliveira, I. S., Stevenson, P. A. and Mayer, G. (2017). The nervous and visual systems of onychophorans and tardigrades: learning about arthropod evolution from their closest relatives. *J. Comp. Physiol. A* **203**, 565-590. doi:10.1007/s00359-017-1186-4

Martínez-Salazar, S., González, F., Alzate, J. F. and Pabón-Mora, N. (2021). Molecular framework underlying floral bilateral symmetry and nectar spur development in Tropaeolum (Tropaeolaceae), an atypical member of the Brassicales. *Am. J. Bot.* **108**, 1315-1330. doi:10.1002/ajb2.1719

Martínez-Salazar, S., Kramer, E., González, F. and Pabón-Mora, N. (2023). Spatio-temporal expression of candidate genes for nectar spur development in Tropaeolum (Tropaeolaceae: Brassicales). *Ann. Bot.* **132**, 1205-1218. doi:10.1093/aob/mcad164

Mayer, G., Franke, F. A., Treffkorn, S., Gross, V. and Oliveira, I. S. (2015). Onychophora. In: *Evolutionary Developmental Biology of Invertebrates* (ed. A. Wanninger), pp. 53-98. Berlin: Springer. doi:10.1007/978-3-7091-1865-8_4

Muñoz-Gómez, S., Suárez- Baron, H., Alzate, J. F., González, F. and Pabón-Mora, N. (2021). Evolution of the subgroup 6 R2R3-MYB genes and their contribution to floral color in the perianth-bearing Piperales. *Front. Plant Sci.* **12**, 633227. doi:10.3389/fpls.2021.633227

Ortíz-Ramírez, C., Giraldo, M., Ferrándiz, C. and Pabón-Mora, N. (2019). Expression and function of the bHLH genes ALCATRAZ and SPATULA in selected Solanaceae species. *Plant J.* **99**, 686-702. doi:10.1111/tpj.14352

Pabón-Mora, N., Suárez-Baron, H., González, F. and Ambrose, B. A. (2015). Flower development and perianth identity candidate genes in the basal angiosperm Aristolochia fimbriata (Aristolochiaceae). *Front. Plant Sci.* **6**, 1095. doi:10.3389/fpls.2015.01095/full

Pabón-Mora, N., Madrigal, Y., Alzate, J. F., Ambrose, B. A., Ferrándiz, C., Wanke, S., Neinhuis, C. and Gonzalez, F. (2020). Evolution of Class II TCP genes in perianth-bearing Piperales and their contribution to the bilateral calyx in Aristolochia. *New Phytol.* **228**, 752-769. doi:10.1111/nph.16719

Pérez-Mesa, P., Ortíz-Ramírez, C. I., González, F., Ferrándiz, C. and Pabón-Mora, N. (2020). Expression of gynoecium patterning transcription factors in Aristolochia fimbriata (Aristolochiaceae) and their contribution to gynostemium development. *Evo Devo* **11**, 4. doi:10.1186/s13227-020-00149-8

Powder, K. E., Milch, K., Asselin, G. and Albertson, R. C. (2015). Constraint and diversification of developmental trajectories in cichlid facial morphologies. *EvoDevo* **6**, 25. doi:10.1186/s13227-015-0020-8

Salazar-Duque, H., Alzate, J. F., Urrea, A. I., Ferrandiz, C. and Pabón-Mora, N. (2021). Comparative anatomy and genetic bases of fruit development in Selected Rubiaceae (Gentianales). *Am. J. Bot.* **108**, 1838-1860. doi:10.1002/ajb2.1785

Salzburger, W. (2018). Understanding explosive diversification through cichlid fish genomics. *Nat. Rev. Genet.* **19**, 705-717. doi:10.1038/s41576-018-0043-9

Santos, M. E., Lopes, J. F. and Kratochwil, C. F. (2023). East African cichlid fishes. *EvoDevo* **14**, 1. doi:10.1186/s13227-022-00205-5

Stolfi, A. and Brown, F. D. (2015). Tunicata. In: *Evolutionary Developmental Biology of Invertebrates* (ed. A. Wanninger), **Vol. 6**, pp. 135-204. Vienna: Springer. doi:10.1007/978-3-7091-1856-6_4

Sunnucks, P. and Tait, N. (2001). What's so interesting about velvet-worm sex? Tales of the unexpected. *Nature* **27**, 60-69.

Suárez-Baron, H., Perez-Mesa, P., Ambrose, B., González, F. and Pabón-Mora, N. (2016). Deep into the Aristolochia Flower: expression of C, D and E class genes in Aristolochia fimbriata (Aristolochiaceae). *J. Exp. Zool. Part B.* **328**, 55-71. doi:10.1002/jez.b.22686

Suárez-Baron, H., Alzate, J. F., González, F., Pelaz, S., Ambrose, B. A. and Pabón-Mora, N. (2021). Gene expression underlying floral epidermal specialization in Aristolochia fimbriata (Aristolochiaceae). *Ann. Bot.* **127**, 749-764. doi:10.1093/aob/mcab033

Suárez-Baron, H., Alzate, J. F., Ambrose, B. A., Pelaz, S., González, F. and Pabón-Mora, N. (2023). Comparative transcriptomic analyses reveal key factors controlling floral trichome development in Aristolochia (Aristolochiaceae). *J. Exp. Bot.* **74**, 6588-6607. doi:10.1093/jxb/erad345

Waddington, C. (1941). Evolution of developmental systems. *Nature* **147**, 108-110. doi:10.1038/147108a0

Woods, H. A. and Moran, A. L. (2020). Reconsidering the oxygen-temperature hypothesis of polar gigantism: successes, failures, and nuance. *Integr. Comp. Biol.* **60**, 1438-1453. doi:10.1093/icb/icaa088