

COMMENTARY

Barnacles, barrier loci and the systematic building of species

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In 1846, Charles Darwin embarked on an endeavour with great ramifications for our understanding of the origins of species (Stott, 2003; Richmond, 2007; Deutsch, 2009). His trip as the naturalist on the *Beagle* had ended ten years earlier, he had had his epiphany about finches, and he had completed his transmutation notebooks, one of which contained his famous sketch of a phylogenetic tree. Thus, Darwin's ideas about descent with modification were well formed. Indeed, in January 1847, Darwin had given his friend Joseph Hooker a 230-page essay of his theory of evolution by natural selection (Barnes & Noble Sparknotes. <http://www.sparknotes.com/biography/darwin/section9.rhtml>). Hooker responded that although the argument was well reasoned, it was not yet convincing. Darwin realized that to make a strong case, he needed to become an expert on a specific group to provide clear evidence for evolution before he generalized to all species. At the time, Darwin had described every specimen from his *Beagle* trip except one, a barnacle, which he would name 'Mr. Anthrobalanus' for its articulated joints, consistent with the discovery that barnacles were crustaceans (Darwin Correspondence Project. <http://www.darwinproject.ac.uk/barnacles>). Thus, beginning with *Anthrobalanus*, Darwin classified barnacles for the next eight years, culminating in two volumes on living and two on fossil *Cirripedia* (Darwin, 1851a, b; Darwin, 1854a, b).

The work on barnacles allowed Darwin to test his views of species as evolving entities connected by lines of common descent (Crisp, 1983). In the process, Darwin developed a new system of natural classification based on homologies and phylogenetic relationships, rather than just using phenotypes as descriptors of similarity in body plan (Ghiselin, 1969; Ospovat, 1981). In his endeavour, Darwin was influenced by Henri Milne-Edwards (1844) and Gaspard Auguste Brullé

(1844) whom, following Karl Ernst von Baer, argued that comparative embryogenesis yielded important information about systematic relationships and that the most characteristic organs in a group were the first to develop during ontogeny (Rachootin, 1984).

Today, students of evolutionary biology aided by high-throughput DNA sequencing are embarked on a similar endeavour as Darwin to discern the nature of species and the speciation process. However, rather than using homologous morphological traits to ascertain phylogenetic relations, we are often using DNA sequencing to conduct genome scans to distinguish 'barrier loci' contributing to reproductive isolation (RI) from loci that do not affect RI. Several excellent recent reviews (Seehausen *et al.*, 2014; Hoban *et al.*, 2016; Wolf & Ellegren, 2017), including that of Ravinet *et al.* (2017) in this issue of JEB, describe in detail the promise and pitfalls of using genome scans to identify barrier loci. We therefore highlight only a few key points.

The first point, as practised by Darwin, and practised and preached by our mentors, is to know thy organism. For Darwin, confirming speciation necessitated an immersion in barnacle anatomy and development. This allowed him to identify homologues and determine how these traits evolved through time to generate new species and reveal phylogenetic relationships. The identification and verification of barrier loci also requires a grounding in natural history. As Ravinet *et al.* (2017) espouse, one must have evidence independent from genome scans concerning gene flow and selection to make a strong case that differentiated regions of the genome reflect divergent selection, rather than being due to other causes (Noor & Bennett, 2009; Cruickshank & Hahn, 2014). Moreover, understanding the key ecological or other axes along which selection is acting allows for more meaningful experimental manipulation, transplant and mapping studies to confirm that outlier regions detected in genome scans are the targets of selection (Anderson *et al.*, 2011a, b; Barrett & Hoekstra, 2011; Soria-Carrasco *et al.*, 2014; Egan *et al.*, 2015; Thurman & Barrett, 2016).

Basic natural history can also lead to big surprises. During his work on barnacles, Darwin discovered minute males parasitic on hermaphrodites in some genera. The finding was unique in animals, leading Darwin to hypothesize that the parasitic males represented a stage in the evolution of distinct sexes (Darwin, 1851a; Darwin, 1854a). The *Timema* stick insects studied by us exhibit colours and patterns that improve crypsis on the host plants upon which they rest (Sandoval, 1994a, b; Sandoval & Nosil, 2005; Sandoval & Crespi, 2008). Similar to mimetic coloration in butterflies (Jiggins *et al.*, 2001; Jiggins, 2008), these colours were long thought to play a critical role in speciation (Nosil, 2007). However, recent work has shown that speciation involves much more than divergence in cryptic coloration, and points to mating isolation and

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other reproductive barriers as being important (Riesch *et al.*, 2017). Mating isolation is based on chemical cues, which are hidden from plain view. Thus, only with careful scrutiny of this chemical world did the dynamics of speciation in *Timema* begin to come clear. Another example studied by us comes from the *Rhagoletis pomonella* sibling species complex, a well-known case of ecological speciation with gene flow via host-plant shifting (Feder *et al.*, 1988). A key trait is diapause life history timing that adapts these flies, including the hawthorn and recently formed apple race of *R. pomonella*, to differences in when their host plants fruit (Filchak *et al.*, 2000). DNA sequence analysis revealed that inversion polymorphism contributing to eclosion time differences has a deep history that can be traced to an isolated population of hawthorn flies in the central highlands of Mexico (Feder *et al.*, 2003). Episodes of gene flow from Mexico into the United States over the last 1.5 million years appear to have infused hawthorn populations in the United States with variation that subsequently played a role in the shifts of the fly to novel hosts. Thus, for barnacles, *Timema* and *Rhagoletis*, important evolutionary plot twists would not have become apparent without immersion in natural history and other details of organismal biology.

Indeed, knowing thy organism goes beyond natural history to encompass the genomic environment. As discussed by Ravinet *et al.* (2017), information on recombination and mutation rates, gene density and architecture, and structural features of the genome (e.g. inversions, translocations, centromeres) is also needed to properly evaluate genome scans for barrier loci. The ecological, demographic/historical and genetic aspects of the study of barrier loci are associated with different schools of evolutionary biology. Naturalists and field-oriented biologists often study extrinsic RI and ecological variables, whereas those focused on molecular evolution and model systems often examine intrinsic isolation and genetic variables. But to resolve how different barrier loci collectively generate RI requires investigators to wear multiple research hats, as Darwin did as both a barnacle taxonomist and embryologist for eight years.

Second, understanding speciation involves more than just identifying barrier loci but also determining how they get put together to form new species. Hunting for a 'speciation gene' is part of the endeavour, as was discovering a homologous trait in barnacles for Darwin. In isolation, however, a homologue or barrier locus may mean little; they assume their significance when placed in the proper context of how and when different phenotypes arise and become associated to form new species (Barton, 1983; Smadja & Butlin, 2011). At the current time, genome scans have been conducted for several individual pairs of taxa, with the pairs generally representing single, nonuniform snapshots in time, coming from a variety of different organismal groups.

Such temporally and taxonomically disjointed data make it difficult to deduce how speciation unfolds within groups and to assess similarities and differences in the process among groups. Relatively few studies have examined population pairs of related taxa at varying stages of divergence along the 'speciation continuum' within a group (reviewed by Seehausen *et al.*, 2014). Such comparisons are needed to more fully understand the processes and dynamics of how barrier loci transition from acting alone and having local effects on genomic differentiation to becoming coupled to collectively act to reduce RI genomewide (Barton, 1983; Barton & De Cara, 2009; Smadja & Butlin, 2011; Feder *et al.*, 2012). It will be interesting to see whether, analogous to Darwin's embryology, generalities emerge concerning the 'ontogeny' of different types of barrier loci among groups. For example, does divergent ecological selection often play a critical role in initiating population divergence and is this related to speciation mode (initial divergence with or without gene flow)? Different stages or types of species may also be recognizable at different points along the speciation continuum (Feder *et al.*, 2012). For example, races may form distinguishable genotypic clusters from each other locally in the landscape, but not globally across their geographic range of overlap. In ecological species, genotypic clusters may be seen across the entire geographic, but not genomic, landscape, with the effects of RI still limited mainly to genes and gene regions under selection. Finally, when taxa more akin to strict biological species co-occur and potentially hybridize, barrier loci may become sufficiently coupled that their indirect effects cause neutral sites throughout the genome and species' ranges to diverge significantly, as well. Coyne (1992, p. 290) noted that, 'It is clear that the arguments [about species concepts] will persist for years to come but equally clear that, like barnacles on a whale, their main effect is to retard slightly the progress of the field. Ultimately, speciation will require less rumination and more perspiration'. The efficacious use of genome sequencing and identification and characterization of barrier loci across the speciation continuum for related taxa with well-resolved natural histories and genetics may lend the perspiration needed to help clarify the species question.

Our third and last point is that, just as Darwin was not afraid to apply new approaches to developing the field of systematics, we may gain by exploring new approaches towards studying speciation. For example, might approaches used to anticipate critical transitions in other complex systems provide new insights into the dynamics of speciation? Studies of the potential of ecosystems, societies and financial institutions to undergo sudden regime shifts from one state to another have suggested some generic features that may in principle affect critical transitions for any complex system (reviewed in Scheffer *et al.*, 2012). Networks in which the components (i.e.

nodes) are heterogeneous and incompletely connected are highly modular, promoting gradual node-by-node adjustment to change. By contrast, in highly connected networks, local losses tend to be 'repaired' by subsidiary inputs from linked units until, at a critical stress level, the system collapses (Scheffer *et al.*, 2012). There are potential parallels here with the coupling of barrier loci and rapid transitions from genic to genomic phases of speciation (Flaxman *et al.*, 2013, 2014; Nosil *et al.*, 2017). Barrier loci may be thought of as the nodes in a genome network connected by recombination, linkage disequilibrium, epistasis, developmental pathways, and the direct and indirect effects of selection. The stronger barrier loci become coupled the stronger the evolutionary feedback and potential for nonlinear divergence dynamics. Nonlinear dynamics do not have to rely on epistatic fitness interactions or physical linkage between genes in a network, however. When effect sizes of mutations are small compared to the migration rate during speciation-with-gene-flow, unlinked variants will initially accumulate at a slow and relatively steady pace, displaying little differentiation between populations (Flaxman *et al.*, 2013, 2014; Feder *et al.*, 2014). However, when a threshold number of divergently selected genes establish, a tipping point can be reached where collectively the combined direct and indirect effects of selection acting on loci become greater than the homogenizing force of migration between populations. At this point, a positive feedback loop is initiated and divergence and linkage disequilibrium will dramatically increase in a nonlinear manner between populations. At this time, the probabilities for new mutations to establish will also elevate, resulting in the differential congealing of the genomes of taxa into distinguishable entities (a phase shift from one to two semi-independent to fully independent genetic networks) that we may recognize as different species (Flaxman *et al.*, 2013, 2014; Feder *et al.*, 2014). Analogous dynamics apply to allopatric speciation with regard to whether sufficient numbers of barrier loci and reproductive isolation has evolved between populations for them to remain and continue to diverge vs. fuse if and when they were to come into secondary contact and hybridize (Barton, 1983; Feder *et al.*, 2013). We finally note that similar transition state rules may also apply to the speciation problem when envisioning nodes as local demes in a meta-population or as species in a community.

In conclusion, we highlight that thinking more broadly about barrier loci in phylogenetic and network contexts, coupled with diligent work resolving the natural history and genetics of systems, holds great promises for revealing new insights about speciation. Through gaining a deep understanding of a study system and applying system approaches, we may come to understand better how species are built and evolve from their component parts (barrier loci), as Darwin did considering morphology and development in

barnacles. Although challenges remain concerning identifying and verifying barrier loci, we see the question of how they become assembled to create new biodiversity (Barton, 1983; Barton & De Cara, 2009; Smadja & Butlin, 2011; Feder *et al.*, 2012) as the outstanding question facing students of speciation.

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