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In 1846, Charles Darwin embarked on an endeavor 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 (1851a, b, 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 endeavor, 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 endeavor 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 practiced by Darwin, and practiced 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 (Barrett & Hoekstra 2011, Anderson et al. 2011a, b, 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 (1851a, 1854a). The Timema stick insects studied by us exhibit colors 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 colors 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 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 US over the last 1.5 million years appear to have infused hawthorn populations in the US 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 endeavor, 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, non-uniform snapshots in time, coming from a variety of different organismal groups. Such temporally and taxonomically disjointed datum points 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 genome wide (Barton 1983, Barton & Cara 2009, Smadja & Butlin 2011, Feder et al 2012). It will be interesting to see if, 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 non-linear divergence dynamics. Non-linear dynamics do not 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 and act independently (fitness interactions are multiplicative) 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 becomes greater than the migration rate between populations. At this point, a positive feedback loop is initiated and divergence and linkage disequilibrium will dramatically increase in a non-linear 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- 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 versus 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 & Cara 2009, Smadja & Butlin 2011, Feder et al 2012) as the outstanding question facing students of speciation.

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