

# Gene Flow Happens

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Debate over what is a species was already considered old hat when Darwin wrote his seminal abstract (as he called it) more than 150 years ago.<sup>1</sup> Endless papers, workshops, and symposia have been presented in an effort to "solve" the species problem. Yet, here we are, at it again. Has there been any progress? I believe that there has been, and that among the many advances enabled by the genomics revolution, progress on species concepts and species recognition is among them. To quote Feder and colleagues,<sup>2</sup> we are on the brink of a "unified theory of speciation genomics."

To claim that the problem is no longer difficult would be absurd. But it is the very difficulty of the problem that lends so much to its fascination. I happen to believe that confronting one's views on the subject is akin to evolutionary psychoanalysis, justifying belief with evidence. Though it is something of a tautology to say that species are the product of speciation, it is useful to remind ourselves that "Speciation is a multi-level process unfolding through time and space."<sup>3</sup> In this vein, de Queiroz<sup>4</sup> has elegantly made the point that by attempting to pin a name on an organismal unit that is the product of an ongoing process, replete with a complex past and an unknown future, we are setting

ourselves up for disagreement. According to this view, it is almost a matter of taste where one decides to draw a line on the graph (see Figure 1 in de Queiroz<sup>4</sup>). No wonder this is so hard. But I take comfort in the philosophical views articulated by Hull,<sup>5</sup> who clarified the essential conflict between the applicability and theoretical relevance of any given species concept. In this view, those concepts that are easily applicable are almost by definition theoretically bankrupt, with the converse also being true. On one hand, one might imagine a species concept wherein any two organisms that are shown to have diverged at the cytochrome *b* locus by 2% or more are diagnosable as species. (Indeed, this happens with uncomfortable regularity.) But what does that tell us about the evolutionary process? Isn't it more than a little subjective? Why not 1.5% divergence, to be more lenient? Or why not require divergence that is greater than 2.5%, to be more rigorous? Under such a criterion, the identification of species is an operational but highly arbitrary exercise. On the other hand Templeton's<sup>6</sup> definition of Cohesion Species as "the most inclusive" population with potential for phenotypic cohesion through intrinsic cohesion mechanisms ... having the potential for genetic and/or demographic exchangeability" has apparent theoretical appeal, but how on earth could

this be applied to the actual identification of species in nature? No clues are given.

My own approach to the problem has been rather workmanlike. I have spent an appreciable amount of my career studying patterns of speciation in mouse lemurs, but not because I necessarily thought of them as an ideal model system for studying speciation more generally. Rather, I have tried to interpret these patterns because I am fascinated by the organisms themselves, the biological diversity that they represent and, most importantly, the evolutionary processes that produced them. My interest in mouse lemurs and their species diversity began with a collaboration instigated by my friend and colleague, Steve Goodman, of the Field Museum of Natural History. In the late 1990s, Steve approached me with an invitation to "prove with genetic data" that the morphological and biogeographic patterns of diversity that he and Rodin Rasoloarison, then a graduate student, were observing in mouse lemurs throughout western Madagascar were, in fact, evidence of unappreciated species diversity.

Given that mouse lemurs, to most eyes, are morphologically homogeneous, I initially assumed that the diversity observed by my colleagues was probably more consistent with intraspecific rather than interspecific divergence. Accordingly, I took a conservative approach by choosing to amplify and sequence a very rapidly evolving region of the genome, the mitochondrial D-loop. At the time, this was a popular locus for analyzing population-level genetic diversity and, given that I was then absolutely certain that the mouse lemur project was going to be one of detecting diversity within a single

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metapopulation, this seemed appropriate. Consequently, I was astonished when the sequence data were so variable among the populations that they could barely be aligned. At that moment, I stepped back and reassessed, and haven't looked back since. The high levels of mitochondrial diversity, their phylogenetic congruence with Rodin's morphometric analyses,<sup>7</sup> and the consequent revision to biogeographic models<sup>8</sup> utterly convinced me that mouse lemur species diversity was seriously underestimated.

But here comes the hard part. Why does any of this matter? And what about answering the questions posed by the editor of this journal: What is a species? How do you identify a species? And finally, what are the implications of one's approach for advancing our appreciation of primate diversity?

My answer to the first question relates more to process than to pattern; to the "whys," rather than the "whats" of evolution. Although recognizing species as measurable units is fundamental to pursuits such as calculating biodiversity indices in the present as well as quantifying faunal assemblages in the past, I personally am more interested in recognizing the units as a means for determining the evolutionary processes that shaped them. Accordingly, I am principally drawn to Simpson's Evolutionary Species Concept, as expressed in Weisrock and coworkers.<sup>9</sup> That is, a "lineage (an ancestral-descendent sequence of populations) evolving separately from others and with its own unitary evolutionary role and tendencies."<sup>10</sup> As for how to identify these lineages as species, I believe that De Queiroz<sup>4</sup> describes it best when he says that "all of the properties formerly treated as secondary species criteria are relevant to species delimitation to the extent that they provide evidence of lineage separation and thus more lines of evidence are associated with a higher degree of corroboration." In other words, the observations I have described regarding genetic distance, morphometric distinction, and biogeographic separation are the lines of evidence supporting my species

recognition hypotheses. With the addition of yet more "lines of evidence," as is increasingly the case with the mouse lemur radiation,<sup>11-13</sup> my confidence has only increased.

But referring back to the first question – "What is a species?" – I must confess to having always been held in the sway of Mayr's Biological Species Concept (BSC).<sup>14</sup> This concept is founded on the principle that true species are reproductively isolated, one from the other, and that the principle mode of speciation is allopatric. Having always imagined that the "moment" of speciation is essentially a point of no return, a time when a single species rather

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suddenly becomes two under the model of a bifurcating evolutionary history, it is somehow reassuring to think of that moment as reinforced by the cessation of gene flow. Indeed, in looking back over the progress of my own work, I find, somewhat to my surprise, numerous claims that the mouse lemur species in question are often found under conditions of "reproductive isolation in sympatry."<sup>8,15</sup> After all, that is the *sine qua non* of speciation. Or is it? Certainly reports of gene flow (that is, introgression) among mouse lemur species are on the rise,<sup>16</sup> yet my certainty that these species are

"good species" remains unshaken. Why is this so?

Increasingly, the theoretical framework for interpreting genomic data in the context of speciation is influencing the way that I think about the problem, as well as the way that I approach the issue of species identification. In the view of some, we are well into a period of a "speciation revolution."<sup>17</sup> It was Wu's paper<sup>18</sup> on the "genic view of speciation" that is considered seminal. The premise is simple: speciation reflects "a process of emerging genealogical distinctness, rather than a discontinuity affecting all genes simultaneously." The process is driven by selection on specific regions of the genome and, thanks to the focus on selection rather than isolation as the driving force, this process provides a general theory that fuses genetics with Darwinian theory.<sup>17</sup> In other words, speciation is not an all or nothing event. Instead, reproductive isolation is frequently incomplete until long after categorical speciation.<sup>19</sup> As Mallet<sup>17</sup> describes this revolutionary and seemingly explicit rejection of the BSC, we are "apparently emerging from a 60-year-old blind alley, a veritable Dark Ages compared with the typical pace of modern science."

Initially, there was a considerable degree of pushback to Wu's Genic Speciation Model. Among the various complaints were that it applies rather strictly to *Drosophila*, and thus is not general;<sup>20</sup> that the BSC is being used as a straw man;<sup>21</sup> and that it is too restrictive, given its sharp focus on selection as the driving force of speciation.<sup>22</sup> However, the theory has gained momentum in the ensuing decade, with empirical data increasingly showing support for the model and, indeed, expanding on it.

Of greatest interest in this regard is the concept of "genomic islands of speciation." Originally formulated in an empirical setting,<sup>23</sup> the concept has been more broadly conceived as a case in which certain regions of the genome (typically, loci under strong selection) show patterns of divergent evolution even in the face of considerable gene flow. Moreover, surrounding areas of the genome, even if evolving neutrally, can show similar patterns of population divergence (as measured by  $F_{ST}$ ) via the

process of divergence hitchhiking (DH). Theoretically, speciation can thus proceed from a stage at which genomic islands are small and dispersed throughout the genome to a later stage at which genome-wide divergence occurs and the genomic islands are erased.<sup>24</sup>

Empirical work appears to be bearing out these theoretical predictions.<sup>25–27</sup> The hypothesized process has been formalized into a four-phase model:<sup>2</sup> Phase 1 = a stage of direct selection acting on specific regions of the genome; Phase 2 = genic divergence via DH; Phase 3 = genomic divergence when the strength of selection acts across the entire genome, thereby driving divergence at all loci via genomic hitchhiking; Phase 4 = postspeciation divergence, wherein populations evolve as if allopatric and without gene flow. (As an interesting side note, Feder and coworkers<sup>2</sup> liken Phase 4 to the Phylogenetic Species Concept, which means that they are thinking about this particular concept very differently than did its originators.)

So where does all this leave us? We are still faced with naming a species that is a time sample of an ongoing, and in some cases, reversible<sup>28</sup> process. Moreover, there are no discrete guidelines for the conclusive identification of genomic islands as they relate to the speciation process.<sup>29</sup> Though genome-scale data are fundamental to the pursuit, the requisite number of individuals within and among species remains ambiguous. Many would consider this no progress at all. I, however, beg to differ. With the new genomic perspective we have an explicit description of organismal characteristics that are measurable and comparable across a large segment of the Tree of Life. Moreover, we have a conception of the process that is equally applicable to both allopatric and sympatric speciation.

Until the very recent past, the ability to generate data of the magnitude necessary to detect whole-genome variation has been beyond the reach of most organismal biologists, and certainly has been so for those of us

who study nonmodel organisms. But with the advent of next-generation sequencing, there has been a profound shift in what is possible. Advances in sequencing technologies and bioinformatics now place in the hands of primatologists the ability to collect, analyze, and interpret genome-wide patterns and apply them to our understanding of speciation, and by extension, our definition of species. Although there will continue to be disagreements among investigators about whether the tipping point has been reached, or even if that tipping point exists, we now have available not only the opportunity to collect data, but the language for discussing the process. I therefore argue that we are as close as we have ever been to reaching a species definition in which theoretical relevance and applicability merge. Thus, to answer the third question posed by the editor, we can now begin to study primate diversity by using measures and methods that are compatible with those of other organismal biologists. Consequently, the question of “lumping versus splitting” should soon become irrelevant.

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