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Title
Wherefore and Whither the Ring Species?

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Wherefore and Whither the Ring Species?

Shawn R. Kuchta[1] and David B. Wake[2]

Ring species are widely recognized as one of the best natural illustrations of species formation. A ring species is a circular arrangement of populations with one boundary characterized by reproductive isolation, but intergradation among populations elsewhere. They form when populations disperse around a central barrier and form a secondary contact characterized by reproductive isolation. Ring species are often presented as a taxonomic conundrum, because the presence of a single boundary exhibiting reproductive isolation leaves the ring of populations uncomfortably situated between one and two species. Here we review the ring species concept, with a focus on the salamander *Ensatina eschscholtzii* and the Greenish Warbler, *Phylloscopus trochiloides*. We argue that ring species demonstrate the gradual nature of species formation, and thereby illustrate the model of species formation originally put forth by Darwin. We also argue that ring species have become overly idealized, with a focus on strict criteria to the detriment of evolutionary lessons. Like all models of evolutionary change, the ring species concept is an oversimplification, and an ideal ring species has never been found. Finally, we review ring species in light of the general lineage concept of species, and argue that ring species status, while nicely accommodated by recognizing a single species, is independent of taxonomy. The essential features of a ring species are a biogeographic history resulting in a ring-like distribution, and the presence of a single species border characterized by reproductive isolation. Under the general lineage concept, reproductive isolation is a contingent, but not necessary, property of evolutionary lineages. Whether one considers a ring species complex to be one species or many does not change the evolutionary message, and the problems (and lessons) presented by ring species do not go away with taxonomic changes.

...we never find two 'subspecies' breeding in the same faunal area....

J. Grinnell, 1904, p. 373

LEONHARD Stejneger, in Jordan (1905), was the first to develop a biogeographic scenario by which two subspecies could coexist, and in doing so took the first step toward refuting Grinnell's (1904) observation that subspecies never breed in the same area. Stejneger's idea was that one could get coexisting subspecies if two or more distributional axes descended from a common ancestor dispersed around either end of a central barrier, only to meet on the other side. In doing so, the two axes could locally coexist if, in the course of expansion, each had diverged to the point of becoming reproductively isolated from one another, and yet they would maintain their conspecific status if the two descendent lineages intergraded with populations in the ancestral part of the range (Fig. 1A). But this scenario was conjecture. Did such a species exist? In 1942, Mayr rose to the challenge and identified nine potential examples. He called them "circular overlaps," and repeatedly stated that they were the "perfect demonstration of speciation" (Mayr, 1942, 1963, 1970). Cain (1954) coined the term "ring species" for such cases. Much empirical research has been done on ring species since Mayr, though two examples dominate the current discussion: the salamander *Ensatina eschscholtzii* (Stebbins, 1949) and the Greenish Warbler, *Phylloscopus trochiloides* (Irwin et al., 2001a).

There is an inherent attraction to ring species as a demonstration of species formation, yet controversy and confusion persist. What makes them the perfect demonstration of speciation? Are ring species a general feature of evolution, reflecting an evolutionarily important process of diversification? Or, as suggested by the limited number of vetted cases, are they a novelty, perhaps nicely illustrating an aspect of species formation, but rare? In this paper we review the ring species paradigm with an eye toward the historical development of the idea. We argue that the ring species concept has drifted in meaning through time, and in doing so has become unnecessarily idealized. Diagnosis of ring species status has trumped a consideration of the evolutionary processes exemplified, and under a strict definition of ring species there are no natural examples. In addition, ring species offend some workers, leading them to urge that ring species be taxonomized out of existence:

> "One of the reasons why [ring species] have not been recorded in the literature more frequently is a purely psychological one. The puzzled systematist who comes across such cases is tempted to 'simplify' them by making two species out of one ring, without frankly telling the facts. Overlapping rings are disturbing to the orderly mind of the cataloguing systematist, but they are welcome to the student of speciation."

Mayr, 1942, p. 180

Indeed, there are a number of systematists who find the current taxonomy of the *Ensatina* complex, with one species and seven subspecies, deeply dissatisfying (e.g., Frost and Hillis, 1990; Graybeal, 1995; Highton, 1998).

Taxonomy notwithstanding, in this paper we conclude that *Ensatina* and the Greenish Warbler are genuine ring species, because they are distributed in a ring with reproductive isolation at the point of terminal overlap between the distributional arms. Other instances of ring species almost certainly remain to be found (Mulcahy and Macey, 2009; Monahan et al., 2012). Ironically, despite Mayr's praise of ring species, we argue that they exemplify Darwin's ideas on species formation better than Mayr's. Finally, we discuss ring species in light of the general lineage concept of species (de Queiroz, 1998). We conclude that the ring species concept relates solely to biogeography and patterns of reproductive

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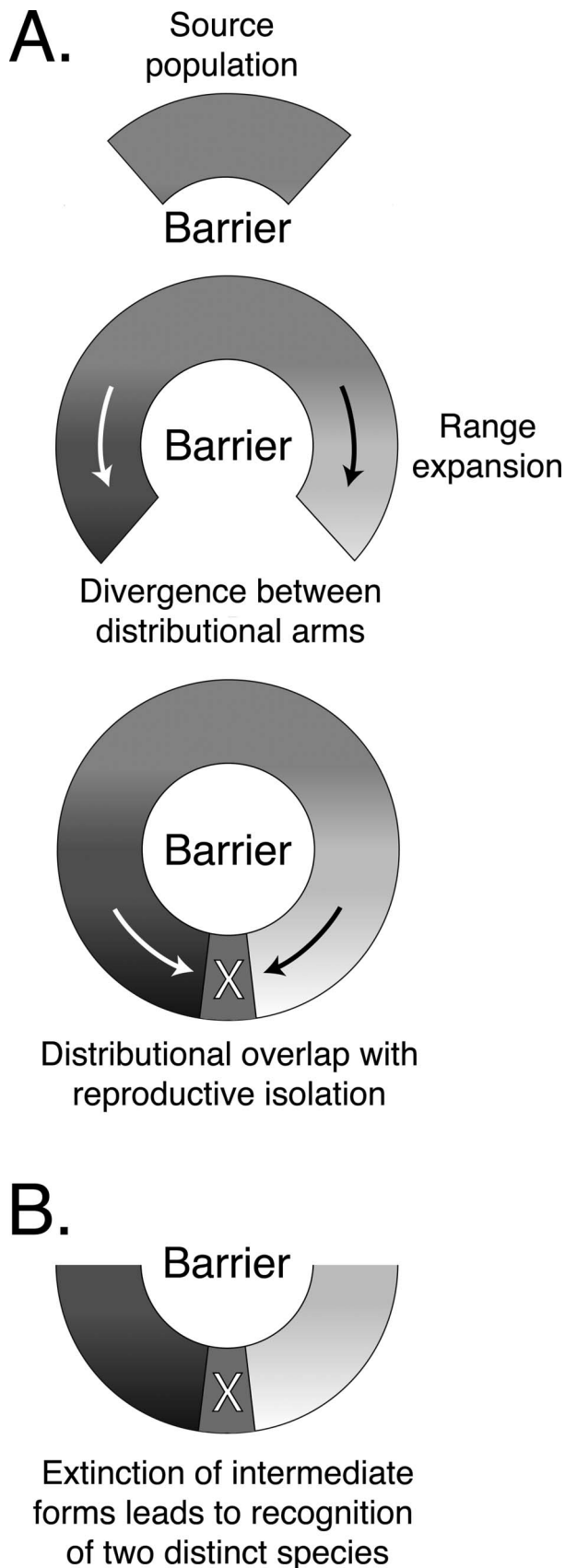
Fig. 1. (A) An idealized diagram of the formation of a ring species. An ancestral source population disperses around a central barrier, the distributional axes adapting and diverging as they expand their range. Upon secondary contact the axes are reproductively isolated, yet are linked to each other by a chain of intergrading forms. (B) The role of extinction in species formation. Even in the absence of continued adaptive evolution, a biotic discontinuity is created by the extinction of intermediate populations.

isolation and is divorced from taxonomy: taxonomic adjustments cannot eradicate a ring species.

A BRIEF REVIEW OF TWO PROMINENT RING SPECIES COMPLEXES

Populations partly separated tended to go their own evolutionary way.

Stebbins, 1949, p. 503

Ensatina eschscholtzii.—The salamander *Ensatina eschscholtzii* is a textbook example of a ring species (Mayr, 1942, 1963, 1970; Futuyma, 1998a; Ridley, 2004). The ring species hypothesis for *Ensatina* salamanders is centered in California, and elements of their life history and population biology mean that California is, for individual salamanders, a very big place. *Ensatina* salamanders are entirely terrestrial, with direct development and no migration (Stebbins, 1954; Collazo and Keller, 2010). Home ranges are small (on the order of a few square meters), dispersal is limited, and territories are defended (Stebbins, 1954; Staub et al., 1995; Wiltenmuth, 1996; Wiltenmuth and Nishikawa, 1998). Territoriality and limited vagility have led to the build-up of high levels of population genetic structure across the range of *Ensatina* (Kuchta et al., 2009a, 2009b; Pereira and Wake, 2009).

The hypothesis that *Ensatina* is a ring species was first advanced by Stebbins (1949) as part of a pioneering analysis that was part taxonomic revision, part evolutionary hypothesis. Stebbins recognized seven subspecies in the complex, including four with a relatively uniform dorsal coloration (the 'unblotched' subspecies *picta, oregonensis, xanthoptica, eschscholtzii*) and three with bright dorsal patches of color overlain on a dark background (the 'blotched' subspecies *platensis, croceater, klauberi*). These seven subspecies are distributed in a ring around the Central Valley of California, an arid region that is currently inhospitable to *Ensatina* salamanders, which require relatively mesic conditions (Fig. 2). In the mountains of southern California the unblotched subspecies *eschscholtzii* and the blotched subspecies *klauberi* are locally sympatric (Stebbins, 1949, 1957; Brown and Stebbins, 1964; Brown, 1974; Wake et al., 1986). A recent detailed study of one of several areas of contact in southern California (Palomar Mountain, San Diego Co.) described a hybrid zone located along an ecotone, with pure parentals and F1s dominating in the region of overlap (Devitt et al., 2011). In a nearby site in the Cuyamaca Mountains (the southernmost known area of contact) no evidence of present or past hybridization is evident, and the two morphologically distinct forms co-occur (Wake et al., 1986). Stebbins (1949) developed a novel biogeographical model to account for this taxonomic oddity of locally sympatric subspecies. He postulated that the *Ensatina* complex originated in present-day northwestern California and southwestern Oregon, perhaps from an ancestor with a *picta*-like phenotype. This ancestral stock expanded southward as two distributional axes down the Coast Ranges (unblotched subspecies) and down the inland ranges (blotched subspecies), the populations adapting and diverging as they spread, until they re-contacted in southern California as reproductively isolated entities (Fig. 2). Broad zones of phenotypic intergradation between adjacent subspecies were interpreted as representative of ongoing genetic connectivity (Dobzhansky, 1958), and the two sympatric subspecies in southern California were
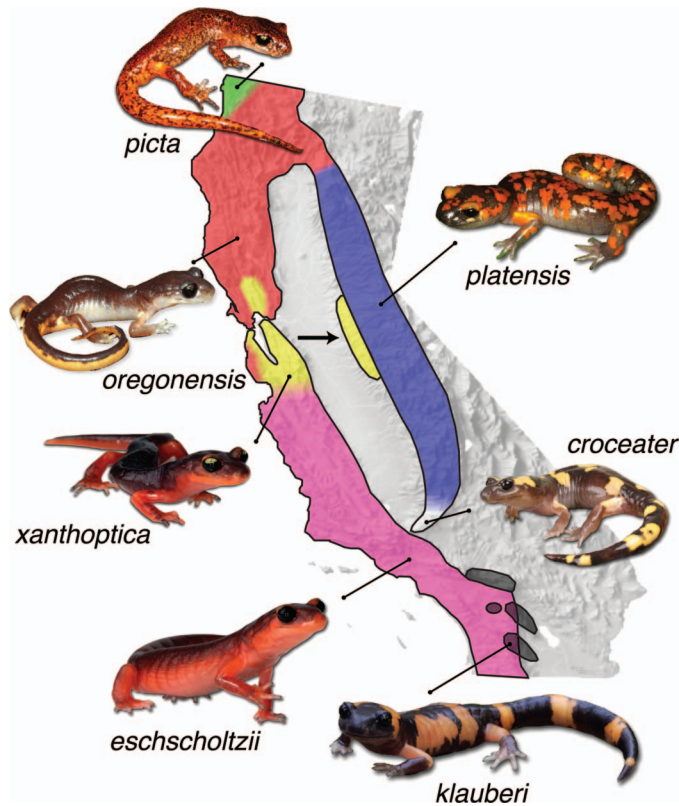
Fig. 2. The distribution of *Ensatina eschscholtzii* in California. The ranges of the seven subspecies are illustrated with different colors, along with representative photographs. Illustrating variation in *Ensatina* is challenging because phenotypic variation is often gradual (Stebbins, 1949) and broader than the genetic transitions (Kuchta et al., 2009a; Pereira and Wake, 2009). On this map, gradations between subspecies illustrate the borders identified by genetic markers. Phenotypic variation is greater than is shown, and many within-subspecies genetic transitions are not identified.

viewed as linked by a continuous sequence of interbreeding populations (Stebbins, 1949).

Stebbins (1949) identified two features of the *Ensatina* complex that complicated its status as an ideal ring species. The first is a geographic break between the blotched subspecies *croceater* and *klauberi* (Fig. 2), which he interpreted as a consequence of recent extinction. The second is a "test" secondary contact midway down the ring, which was the result of dispersal of coastal *xanthoptica* (unblotched) into the foothills of the Sierra Nevada, where it hybridizes with resident *platensis* (blotched; Fig. 2). Levels of interbreeding suggest that reproductive isolation is not nearly so complete in the Sierra Nevada as in the southern contact between *eschscholtzii* and *klauberi*, and this mid-ring contact was thus viewed as consistent with the ring species scenario (Wake et al., 1989). In summary, despite the imperfections, Stebbins saw in *Ensatina* a compelling example of a ring species, as it included divergence along both distributional arms, a mid-ring contact showing that hybridization was still possible, and sympatry of subspecies in southern California (Stebbins, 1949).

Much molecular systematic work has been done on the *Ensatina* complex since Stebbins (1949). Wake and Yanev (1986) were the first to examine patterns of genetic variation. Using allozyme data, they found surprisingly high levels of genetic variation, indicating a multifaceted biogeographic history. For example, the northern portion of *platensis*

differed markedly from the other blotched forms, including southern *platensis*. This is of interest because the coloration of *platensis* is distinctive (Fig. 2), and prior to molecular studies there was no reason to expect the taxon to be composed of distantly related evolutionary lineages. Most importantly, Wake and Yanev (1986) found the highest levels of genetic divergence were between the distributional arms in the south, consistent with the predictions of Stebbins' hypothesis. On the other hand, they rejected Dobzhansky's (1958) hypothesis that gene flow functioned as a glue holding the complex together. Jackman and Wake (1994) next conducted a more finely sampled study of protein variation in *Ensatina* that included all of the inland subspecies, as well as *picta* and *oregonensis* in northern California. Strong patterns of isolation by distance (IBD) were revealed, and they estimated that the genetic break between *croceater* and *klauberi* was no larger than expected given isolation by distance, supporting the hypothesis of a recent extinction of intervening populations (but see Highton, 1998). In addition, a relatively large, geographically localized genetic break was documented between *oregonensis* and northern *platensis* in the Lassen Peak area, at the north end of the Sierra Nevada (Jackman and Wake, 1994). On the other hand, genetic distances are lower (and more alleles are shared) between northern *platensis* and *oregonensis* than between northern and southern *platensis*. Efforts to localize a contact zone between northern *platensis* and *oregonensis* were unsuccessful due to a long history of glaciation and volcanism restricting available habitat, as well as recent repeated logging in the area.

Phylogeographic studies using mitochondrial DNA were initiated by Moritz et al. (1992), who examined cytochrome *b* sequences sampled throughout the range of the species. Their results supported the ring species scenario in that separate coastal (*xanthoptica, eschscholtzii*) and inland (southern *platensis, croceater, klauberi*) clades were identified. In their best-estimate phylogeny, these two clades were sister taxa, with many northern lineages of *oregonensis*, as well as northern *platensis*, originating at the base of the tree. Consistent with earlier allozyme studies, Moritz et al. (1992) found northern *platensis* to be more closely aligned with *oregonensis* than with southern *platensis*.

A second phylogeographic study by Kuchta et al. (2009a) followed Moritz et al. (1992), but with denser geographic sampling. Their goal was to revisit the biogeographic scenario of Stebbins (1949), with a focus on the geomorphological evolution of the California Coast Range system. In short, molecular divergence time estimages suggest that the Coast Ranges of California, which rose out of the Pacific Ocean as a consequence of interactions between the North American and Pacific Plates (Hall, 2002), are younger than the *Ensatina* complex. This creates obvious problems for Stebbins' original biogeographic scenario. Wake (1997) postulated that connections between islands and the mainland were repeatedly forged and broken, providing an opportunity for dispersal and differentiation of the unblotched lineages of *Ensatina*. Today these islands are incorporated into the California Coast Ranges. Phylogenetic tests of competing biogeographic models supported the ring species scenario of Stebbins, as modified by Wake (1997).

Ring closure is fundamental to the ring species concept (Patten, 2010), and a number of studies have examined secondary contacts between the inland and coastal distributional arms. These studies support the original proposition of Stebbins (1949), which was that interbreeding is much more

pervasive in the mid-ring contact between *xanthoptica* and *eschscholtzii* (Brown, 1974; Wake et al., 1989; Alexandrino et al., 2005; Pereira and Wake, 2009) than it is in the terminal contact between *klauberi* and *eschscholtzii* (Brown, 1974; Wake et al., 1986; Devitt et al., 2011). Both regions of contact involve what would be considered species-level entities by most criteria, except an extreme version of the biological species concept that does not allow for any interbreeding at all. A number of secondary contacts within the coastal and inland distributional arms have also been identified. A particularly large amount of phylogeographic diversity among populations was found in the San Francisco Bay area of central California, including three subspecies and ten mtDNA clades (Wake, 1997; Kuchta et al., 2009b; Pereira and Wake, 2009). In contrast with points of ring closure, the highest levels of interbreeding are found between secondary contacts within the distributional arms, such as between northern and southern *platensis*, or between *xanthoptica* and *oregonensis* (Wake, 1997; Pereira and Wake, 2009; Pereira et al., 2011). However, contacts within the distributional arms are not as thoroughly studied as the points of ring closure, and more detailed investigations of contacts within the distributional arms would be a fruitful avenue of research.

In addition to biogeography and reproductive isolation, there is an adaptive component to differentiation within *Ensatina* (Stebbins, 1949; Wake, 2006). One cannot observe the various subspecies without noting the striking differences in coloration, which range from subdued to almost gaudy (Fig. 2). Stebbins (1949) primarily employed coloration in his original development of the ring species hypothesis. His descriptions of color pattern diversity were accurate, including broad zones of color pattern intergradation where genetic data indicate a relatively sharp transition. Blotched subspecies, despite the bold patterning, were hypothesized by Stebbins (1949) to be camouflaged via disruptive coloration (Kuchta, unpubl. data). Unblotched subspecies were hypothesized to be either camouflaged by way of background matching, or to be Batesian mimics of highly toxic, aposematic newts (genus *Taricha*; Stebbins, 1949; Kuchta, 2005; Kuchta et al., 2008). The presence of three types of antipredator coloration in a single species complex is extraordinary. Pereira et al. (2011) examined patterns of reproductive isolation throughout the ring complex by making inferences from patterns of allozymic divergence and found that levels of genetic differentiation better predicted reproductive isolation than did color pattern differentiation. In general, the many studies by Wake and collaborators indicate that the phenotypes represented as subspecies in *Ensatina* are a consequence of local adaptation. Several of the subspecies, despite their phenotypic similarity, are not monophyletic (*platensis, oregonensis,* possibly *picta*), and/or include substantial phylogenetic structure (*eschscholtzii, xanthoptica,* southern *platensis,* multiple clades of *oregonensis*). The phenotypes identified by the subspecies likely reflect environmental conditions and the corresponding selective pressures, not phylogeographic history, and are not necessarily incipient species.

Phylloscopus trochiloides.—The Greenish Warbler, *Phylloscopus trochiloides,* is not a plethodontid salamander. We review it briefly here because it is the other prominent example of a ring species, and there are many important similarities between *Ensatina* and *P. trochiloides.*

The Greenish Warbler is a small, insectivorous bird that breeds in forests of the Palearctic. The species is younger and has a simpler history than the *Ensatina* complex, and it is the new favorite textbook example of a ring species (Futuyma, 2013; Herron and Freeman, 2013; Zimmer and Emlen, 2013). The original hypothesis that the Greenish Warbler is a ring species traces back to Ticehurst (1938) and Mayr (1942). Six subspecies are recognized, five of which (*viridanus, ludlowi, trochiloides, obscuratus,* and *plumbeitarsus*) form a ring around the treeless, inhospitable (for Greenish Warblers) Tibetan Plateau of Asia (Irwin et al., 2001b, 2005). The complex was hypothesized to have originated in the Himalayas and, following the last glacial cycle, spread north around the east and west sides of the Tibetan Plateau. In Siberia the two distributional axes, represented by the subspecies *viridanus* and *plumbeitarsus*, came into secondary contact, but failed to interbreed freely. A distributional gap on the eastern side of the ring was interpreted as due to recent extinction, probably human mediated, and genetic studies using amplified fragment length polymorphisms (AFLPs) were consistent with isolation by distance and gradual genetic divergence throughout the ring (Irwin et al., 2005; Martins et al., 2013). Finally, studies of song structure showed that songs in the south are short and simple, whereas songs in the north, where the terminal forms *viridanus* and *plumbeitarsus* meet, are relatively complex (Irwin et al., 2001b, 2008; Kovylov et al., 2012). This is important because songs play a key role in species recognition, and while songs in the north are complex, complexity has been achieved in divergent manners. In the western form (*viridanus*), songs are composed of long syllables with a large frequency range. In the eastern form (*plumbeitarsus*), songs are composed of several short syllables with a smaller frequency range. Irwin and colleagues argue that the eastern and western distributional axes experienced similar ecologies, including sexual selection for song complexity, and that parallel selection pressures combined with historical contingencies led to differentiated songs and reproductive isolation. If accurate, this illustrates that divergent selection is not required for the adaptive evolution of reproductive isolation.

While the Greenish Warbler is widely considered a prime example of a ring species, an alternative interpretation of the data has always plagued the ring species scenario: phylogenetic evidence using mtDNA identified not one ancestral source to the south of the Tibetan Plateau, but rather two ancestral sources to the east and west (Wake, 2001; Coyne and Orr, 2004). This hypotheses has recently been supported using a large data set of single nucleotide polymorphisms (SNPs; Alcaide et al., 2014). There is also evidence of distinct genetic clusters within the eastern and western segments of the ring. The new biogeographic explanation is that eastern and western lineages of the Greenish Warbler expanded to meet in the north and the south. In the south, the lineages lacked reproductive isolation and are in the process of merging (individuals in this transitional area are assigned to the subspecies *ludlowi*). In the north, the expanding distributional arms exhibited reproductive isolation and now interact as distinct species (Irwin et al., 2001a). The genetic data of Alcaide et al. (2014) indicate that there has been some interbreeding between the terminal forms in the north, though not enough to promote merger or challenge the interpretation that this is a species-level border. In summary, the history of *P. trochiloides* is like *Ensatina* in that it is characterized by periods of allopatric differentiation, a distributional gap in the ring, and limited gene flow between the terminal taxa. In our view, the Greenish Warbler remains an excellent example of a ring species.

WHAT DO WE LEARN FROM RING SPECIES?

What is a difficulty to the cataloguing systematist is a blessing to the evolutionist.

Dobzhansky, 1958, p. 48

Prior to the Evolutionary (or Modern) Synthesis, a diversity of views on species formation were entertained. While some advocated species formation by way of geographic isolation (e.g., Jordan, 1905; Mayr, 1980), many non-Darwinian mechanisms of species formation were in favor, including orthogenetic and saltational theories (Bateson, 1894; de Vries, 1910; Goldschmidt, 1940; Mayr and Provine, 1980). It was not until Dobzhansky's (1937) and Mayr's (1942) seminal publications that speciation theory took a largely modern form. Mayr's version of the biological species concept was widely adopted, with reproductive isolation serving a dual role as concept and criterion, and species formation was viewed as a gradual process initiated in allopatry. Mayr (1942) referred to this as "geographic speciation," which states that in sexually reproducing animals "a new species develops when a population that is geographically isolated from the other populations of its parental species acquires during this period of isolation characters that promote or guarantee reproductive isolation after the external barriers break down" (p. 155).

It is against the backdrop of the Evolutionary Synthesis that Mayr conceived of ring species as the perfect demonstration of speciation. Mayr defined ring species as a "situation in which a chain of intergrading subspecies forms a loop or an overlapping circle, of which the terminal forms no longer interbreed, even though they coexist in the same localities" (Mayr, 1942:180; Fig. 1A). His focus was on the biogeography of diversification within the geographic speciation framework. The difficulty with studies of geographic speciation is that changes in the geographic distributions of populations and the evolution of reproductive isolation are time-extended processes, whereas most studies of species formation by necessity focus on a single snapshot in time. Mayr reconstructed species formation by arranging studies of diverse taxa into a generalized chronological sequence, similar to an ontogenetic series, from weakly diverged populations on one hand, to sympatric sister species on the other. Ring species are exceptional because, in a single slice of time, they exhibit an array of stages endemic to species formation.

Ever since Mayr (1942), conceptualizations of the value of ring species have shifted to highlight different features of the evolutionary process. This has led to confusion over what ring species exemplify and to disagreements over whether postulated ring species are "real." Below we briefly review different perspectives on the ring species concept. These perspectives are overlapping, yet differ in their emphases.

Geographic speciation.—For Mayr, the merit of ring species was that they came closest to illustrating the entire process of geographical speciation, with space functioning as a surrogate for time. Some argue that Mayr advanced ring species as an example of species formation despite ongoing gene flow (Irwin et al., 2001a; Coyne and Orr, 2004; Liebers et al., 2004; Price, 2008; Patten and Pruett, 2009). Indeed, Mayr described ring species as "speciation by force of distance." The balance between gene flow and selection was not Mayr's intended concern, however. Mayr was a harsh critic of sympatric speciation precisely because he thought gene flow would nearly always overwhelm selection and prevent the evolution of isolating mechanisms (Mayr, 1942, 1963, 1970, 1982). In ring species, distance combined with limited dispersal functioned to restrict gene flow to the point that the terminal axes of the ring had the capacity to evolve independently, as if they were allopatric, while their connectivity through intermediates constituted evidence of their shared ancestry and conspecific status. That ring species were intended to illustrate the process of geographic speciation is well illustrated by the cases presented in Mayr (1942): of the nine examples, three involved taxa distributed among island complexes, and one (*Larus argentatus*) included transoceanic dispersal. Continuous demographic connectivity (an unbroken ring) was not an initial criterion for ring species status.

Species as closed genetic systems.—This idea stems from Dobzhansky (1958), who viewed species as "genetically closed systems" and "the most inclusive Mendelian population." The species category is thus circumscribed by the capacity to interbreed. "They [the reproductively isolated entities at the terminal ends of a ring species, in this case with reference to the *Ensatina* species complex] *can* exchange genes, not directly but by a long circuitous route, through the other races" (Dobzhansky, 1958:49, his emphasis). Species formation here is the splitting of gene pools into separate closed genetic systems. This is the perspective adopted by Dobzhansky and Spassky (1959), who investigated a potential ring species complex in *Drosophila paulistorum*. To examine reproductive interactions, they collected strains from Central and South America and conducted mating trials in the lab. Several strains proved to be reproductively isolated, yet were compatible with a "transitional" strain, showing that novel mutations could spread through the *D. paulistorum* complex. In contrast with Mayr, the study did not identify an ancestral source population, and did not develop a biogeographic scenario involving dispersal around a central barrier.

In a similar spirit, one could argue that ring species highlight nontransitive reproductive interactions with a biogeographic twist. Reproductive compatibility is nontransitive when A can interbreed with B, and B can interbreed with C, but C cannot interbreed with A. The units A, B, and C thus form a single closed genetic system. Nontransitive reproductive interactions do not require a ring-like distribution (Kopp and Frank, 2005; Peterson et al., 2013), but the biogeographic scenario implicit in ring species illustrates how such a situation may evolve.

Species formation despite ongoing gene flow.—Many authors have suggested that ring species show how the evolution of reproductive isolating mechanisms can take place despite the homogenizing influence of gene flow (Irwin et al., 2001b; Liebers et al., 2004; Price, 2008; Patten and Pruett, 2009; Alcaide et al., 2014). The balance between gene flow and natural selection is a major topic in speciation biology (Hendry et al., 2007; Niemiller et al., 2008; Seehausen et al., 2008), and if ring species exemplify this balance they are poised to make a key contribution. That gene flow around a ring of populations could be sufficient to constrain the evolution of reproductive isolation among terminal forms has yet to be established, however. Such a situation would likely require a smaller ring, or higher dispersal, than one finds in any currently recognized ring species, including

Ensatina salamanders and the Greenish Warbler (Wake and Yanev, 1986; Irwin et al., 2005).

The microevolution of adaptive radiation.—Many have proposed that ring species present a convincing demonstration of how intraspecific adaptive evolution accrues, from differentiated populations to subspecies to species (Irwin et al., 2001b; Kuchta et al., 2009a; Patten and Pruett, 2009; Pereira and Wake, 2009). This is because in ring species one finds, in one snapshot in time, multiple levels of divergence, from the initial stages of differentiation to reproductive isolation. In effect, space functions as a surrogate for time, with the intermediate stages in the diversification process preserved. This view of ring species as recording multiple stages in the process of species formation exemplifies Darwin's ideas on species formation. Darwin believed that populations evolved gradually, as divergent selection and other factors led to increased differentiation. Along these lines, the celebrated tree diagram in *The Origin* was not presented so much as a phylogenetic theory of evolution as an explanation of how gradual divergence and extinction can, given sufficient time, lead to the evolution of biotic discontinuities. We discuss Darwin's ideas on species formation more below (in *Species Formation: Darwin vs. Mayr*).

DIAGNOSING RING SPECIES

> *But do ring species exist? A convincing case must meet several criteria.*
>
>   Coyne and Orr, 2004, p. 103

Researchers have focused on different take-home messages when considering ring species. Some have presented ring species as consummate illustrations of species formation, while others have questioned their very existence (Coyne and Orr, 2004; Martens and Päckert, 2007). It is fair to question the reality of ring species, but the answer depends on what a ring species is meant to be. Diverse criteria have been proposed for their diagnosis (Mayr, 1942, 1963; Irwin et al., 2001b; Irwin and Irwin, 2002; Coyne and Orr, 2004; Patten and Pruett, 2009; Cacho and Baum, 2012). Below, we provide a brief review of the criteria that have been used to identify ring species, with a focus on comparing *Ensatina* and the Greenish Warbler to an ideal ring species.

1. Are the populations distributed in an unbroken ring?—An ideal ring species would be continuously distributed across space, without distributional gaps. However, the best-known ring species do not form unbroken rings, but display gaps as a consequence of either the extinction of intermediate forms or long distance dispersal (Mayr, 1942, 1963; Jackman and Wake, 1994; Irwin et al., 2001a; Mulcahy and Macey, 2009). In Mayr's (1942) treatment, many "circular overlaps" were not continuously distributed, but were rings broken by dispersal events. The expectation that a ring species possess an unbroken chain of populations is thus a modern construct, and, as pointed out by Mayr (1963), an unnecessary idealization of the ring species concept (see *Species Formation: Darwin vs. Mayr* below).

2. Is there a point of terminal overlap exhibited by complete (or nearly complete) reproductive isolation, with no reproductive isolation elsewhere?—Reproductive isolation is a broad concept that includes prezygotic barriers, such as ecological or behavioral isolation, as well as postzygotic barriers, such as reproductive incompatibility or hybrid inviability/sterility

(Dobzhansky, 1937; Mayr, 1942, 1963; Coyne and Orr, 2004). The overlap of terminal forms is the *sine qua non* of ring species, and all researchers agree it is necessary that the terminal forms exhibit a high degree of reproductive isolation. An ideal ring species would display complete reproductive isolation. In reality, small amounts of hybridization between the terminal forms are tolerated (Pereira and Wake, 2009; Devitt et al., 2011; Alcaide et al., 2014), which is consistent with how species are commonly diagnosed under the biological species concept (Coyne and Orr, 2004).

3. Are the terminal forms linked by intergrades?—Save for the point of terminal overlap, an ideal ring species would exhibit gradual intergradation among forms around a ring of populations, with no sharp breaks. Most empirical examples of ring species find that the terminal forms are connected by gradual geographic variation, although commonly some regions show more rapid change than others (Stebbins, 1949; Wake, 2006; Irwin et al., 2001a). Relatively sharp genetic transitions in the chain of populations have been documented in *Ensatina* and the Greenish Warbler. Indeed, evidence suggests that the *Ensatina* ring complex may have been broken and re-assembled multiple times (Wake, 1997), which is likely a common feature of rings around long-standing barriers.

4. Is there evidence of a single common ancestor?—An ideal ring species is created when a common ancestor gives rise to two distributional axes that each disperse around a geographic barrier, only to meet on the far side of the barrier (Patten, 2010). Most postulated examples of a ring species include such a biogeographic hypothesis (Mayr, 1942; Irwin et al., 2001a; Irwin and Irwin, 2002). The biogeographic history of a species can be tested using phylogeographic methods, with the expectation that tree topologies are consistent with independent stepwise colonization by two distributional axis, and with each axis originating from a shared ancestral source (Moritz et al., 1992; Kuchta et al., 2009a; Patten, 2010). Note this does not mean that the common ancestor is frozen in time, as populations inhabiting the present-day location of the common ancestor may have evolved away from the ancestral condition.

5. Is there isolation by distance such that the terminal forms are genetically connected through a chain of populations?—An ideal ring species would exhibit isolation by distance throughout the ring of populations, with but a single genetic break where the terminal forms overlap. Nonetheless, there are no examples of ring species that do not exhibit a break somewhere else in the ring, either as a distributional gap or as a secondary contact, or both. Presently continuous rings may have been assembled from allopatric parts, perhaps multiple times, or a ring that is currently broken may have formed a continuous ring in the past. It is possible for an adaptive allele to spread throughout a ring species in the absence of perpetual demographic connectivity if segments of the ring are broken and reassembled through time (Dobzhansky, 1958). For instance, Jackman and Wake (1994) hypothesized that the blotched color pattern in *Ensatina* evolved in the south and spread northward by way of natural selection across a current distributional gap. What we now call northern *platensis* shares a distinctive blotched phenotype with southern *platensis*, while allozyme and mtDNA data suggest that northern *platensis* is more closely related to *oregonensis*. In addition, lightly blotched individuals where

platensis and *oregonensis* meet is suggestive of gene flow and the potential for the blotched pattern to continue to spread, were it to improve fitness in the forests of northern California, even though genetic evidence indicates there is a break in the ring where northern *platensis* and *oregonensis* meet (Stebbins, 1949; Jackman and Wake, 1994).

6. Is there evidence of constant historical connectivity?—The ideal ring species would evolve, then persist in a state of limbo as a consequence of the balance between gene flow, genetic drift, and natural selection. However, there are no known instances of ring species in which the string of populations has maintained constant demographic connectivity. Constant connectivity is unrealistic because climatic cycles and the shifting ranges of ecological communities should restrict the time available for the evolution and maintenance of ring species. Older species, or those that evolve reproductive isolation slowly, such as salamanders (Highton and Peabody, 2000; Wake, 2006; Tilley et al., 2013), are more likely to form rings that have experienced episodes of fragmentation, isolation, differentiation, then expansion and secondary contact (Wake, 1997).

7. Did reproductive isolation evolve despite the homogenizing influence of gene flow around the ring?—When Mayr described ring species as exemplifying "speciation by force of distance," he meant that distance functioned to restrict gene flow to the point that the terminal axes of the ring were effectively allopatric. There currently is no evidence in any ring species that gene flow has had more than a negligible homogenizing influence on the evolution of reproductive isolation between terminal forms. To wit, ring species do not exemplify how reproductive isolation can evolve despite the homogenizing influence of gene flow around a ring.

Most workers have directed attention to criteria 1–4; criteria 5–7 are stricter. No single supposed ring species fulfills all of these criteria, or even criteria 1–4 (thus Coyne and Orr's skepticism regarding the existence of ring species). In our view, too much attention is directed toward evaluating criteria in the hunt for an ideal ring species, when the purpose is to make inferences about species formation. To this end, we begin by comparing Darwin and Mayr's contrasting views on species formation.

SPECIES FORMATION: DARWIN VS. MAYR

It is immaterial whether these instances of circular overlap present themselves in the simplest and most diagrammatic manner or whether they are somewhat more complicated...In either case the process of geographic speciation can be followed step by step. A more dramatic demonstration of geographic speciation cannot be imagined than cases of circular overlap.

Mayr, 1963, p. 510

Although much of the Evolutionary Synthesis concerned developing a genetically informed version of Darwinism, Darwin's views on species and species formation were not widely adopted. One of the great ironies of evolutionary biology is that publication of *On the Origin of Species* is commonly deemed unsatisfactory with regard to how species originate. Mayr, who is credited with best addressing the species problem during the Evolutionary Synthesis, long maintained that Darwin failed to successfully address the origin of species:

"Any pronounced evolutionary change of a group of organisms was, to [Darwin], the origin of a new species. He was only mildly interested in the spatial relationships of his incipient species and paid very little attention to the origin of the discontinuities between them. It is thus quite true, as several recent authors have indicated, that Darwin's book was misnamed, because it is a book on evolutionary changes in general and the factors that control them (selection, and so forth), but not a treatise on the origin of species." (Mayr, 1942, p. 147).

Mallet (2008a, 2010), Reif (2008), and de Queiroz (2011) analyzed the differences between Mayr's and Darwin's answers to species formation and concluded that Darwin did, in fact, have a coherent view of species and their origin. Darwin—who, it should always be remembered, was arguing against special creation—maintained that species were not endowed with any sort of Platonic essence. No single, consistent defining character could be used to distinguish species from varieties, including reproductive isolation, which Darwin explicitly considered and rejected. The process of species formation was viewed through an ecological lens, with persistent natural selection playing a critical role in the gradual evolution of biotic discontinuities. This differs from modern studies of so-called ecological speciation in its lack of focus on reproductive isolation, and in its presumed tempo (Schluter, 2000; Nosil, 2012). Whereas Darwin advocated weak selection and gradualism, modern studies of natural selection and ecological speciation commonly report strong selection and relatively rapid rates of evolution (Hendry et al., 2007; Calsbeek et al., 2012; Thompson, 2013). Most importantly, to Darwin species were not different from varieties, except by being more "strongly marked," and by lacking intermediate forms. He treated the species category as a taxonomic rank, and did not clearly distinguish between the theoretical concept of species and the operational criteria used for species diagnosis (de Queiroz, 2011).

"Finally, then, varieties have the same general characters as species, for they cannot be distinguished from species,—except, firstly, by the discovery of intermediate linking forms, and the occurrence of such links cannot affect the actual characters of the forms which they connect; and except, secondly, by a certain amount of difference, for two forms, if differing very little, are generally ranked as varieties, notwithstanding that intermediate linking forms have not been discovered; but the amount of difference considered necessary to give to two forms the rank of species is quite indefinite." (Darwin, 1859, p. 58–59).

In general, Darwin's leitmotif on species was that they were the end-product of evolutionary processes, including extinction, that resulted in biotic discontinuities. For example, if the intermediate linking populations in a ring species were to become extinct, Darwin (as well as many modern systematists) would have categorized the terminal forms as separate species (Fig. 1B).

Mayr had a different perspective on the nature of species that is well known to most biologists. For Mayr, species were "groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other

such groups" (Mayr, 1942). He regarded geographic isolation as fundamental to species formation, because in his view reproductive isolation could not evolve in sympatry. In general, Mayr thought speciation was not easy. As Mallet (2008b) put it:

> "...Mayr was seduced by a beautifully symmetrical pair of ideas that dovetailed in apparently perfect Yin-Yang harmony: (1) species cannot coexist unless they are reproductively isolated and (2) a lack of coexistence (allopatry), is necessary for the origin of species." (p. 12–13)

> "...Mayr chose to support the idea that species were fundamentally different from varieties with special characteristics (reproductive isolation), and that they required special evolutionary conditions (e.g. geographic isolation, genetic revolutions) for their divergence, which subspecific evolution did not require. Speciation became more difficult than could be achieved by simple adaptation and character evolution, which Darwin believed was the key to diversification." (p. 14)

We review this history because, in our view, ring species are excellent examples of Darwinian species formation. As Coyne and Orr (2004) observed, an ideal ring species is not a compelling example of geographic speciation because, by definition, the terminal forms did not evolve in allopatry. Rather, ring species illustrate gradual species formation, with space functioning as a surrogate for time. The novelty of ring species is that they capture multiple aspects of divergence, from variation among populations, to isolation by distance, to phenotypic divergence, to reproductive isolation. In a single slice of time, they preserve those stages of gradual divergence normally associated with species formation.

RING SPECIES AND THE GENERAL LINEAGE CONCEPT OF SPECIES

In many areas of biology, species are treated as natural "kinds" in an almost biblical sense.

Wake, 2009, p. 336

Speciation is easy!

Mallet, 2008b, p. 2971

For decades a vexing controversy surrounded the definition of the term 'species,' with widespread disagreement over contradictory species concepts (Ereshefsky, 1992; Mayden, 1997; de Queiroz, 1998). The debate makes sense in that the different concepts are founded on different properties of interest to biologists with different concerns. Nonetheless, if general agreement about the nature of species is lacking, how can one study species formation? In recent years the debate over the species category has eased as the "general lineage concept" (GLC) has become widely acknowledged as providing clarity on the definition of species (de Queiroz, 1998, 1999; see also Wiens, 2004; Weisrock et al., 2010; Camargo and Sites, 2013). Under the GLC, species are separately evolving metapopulation lineages. It is not yet another species 'concept,' but a generalization that subsumes the others: all contemporary species concepts adopt the view of species as segments of metapopulation-level evolutionary lineages (de Queiroz, 1998). By contrast, most contemporary species "concepts" are not concepts at all, but criteria. These criteria—for example, monophyly, niche differentiation,

reproductive isolation, diagnosability, morphological distinctiveness, and so forth—are *contingent* (but not necessary) properties of evolutionary lineages. They are secondary properties of species, the attributes that separately evolving lineages acquire as they change through time.

The GLC represents a solution to one source of conflict over species: that contrasting species concepts can create incompatible taxonomies. For example, under the Biological Species Concept (BSC) one might recognize one set of species, whereas under the Ecological Species Concept one might recognize an entirely different set of species. This problem is avoided if one defines species as segments of metapopulation-level evolutionary lineages, and treats this as the only *necessary* property of species, a perspective that de Queiroz (2005a) refers to as the unified species concept (USC). Under the USC, the secondary properties of species do not define species, yet they remain critical to the study of taxonomy and species formation. First, they provide important evidence for empirically assessing the separation of metapopulation-level lineages. Indeed, systematists are encouraged to consider multiple lines of evidence, as no secondary property is foolproof, and any could fail to correctly separate evolutionary lineages for a complex array of realistic biological reasons. Multiple lines of evidence that are consistent with one another corroborate a hypothesis of lineages separation, while conflicting lines of evidence call the hypothesis of lineage separation into question (McKay and Zink, 2015). Second, under the USC the secondary properties of species can be used to define classes of species, or subcategories of the general species category, such as reproductively isolated species, monophyletic species, and diagnosable species (de Queiroz, 2005b, 2007). The contingent properties of species definitions are fundamental biological attributes and are important because of what they teach us about adaptation, community assembly, formation of the tree of life, the evolution of phenotypic discontinuities, and other important biological topics. In the case of ring species, the contingent property of interest is reproductive isolation.

The USC is unifying in that it is founded on a property common to all contemporary species definitions: species as segments of metapopulation-level evolutionary lineages. Under this view, disagreements about species boundaries will not stem from disagreement over the concept of species. Given the importance of the species category for all of biology, this represents a welcome step forward. On the other hand, the USC is not a panacea for disputes over taxonomy. Disagreements will revolve around the reliability of different methods and kinds of data for lineage diagnosis, problems related to temporal scale (metapopulations that are independent over years or decades may not be independent over thousands or tens of thousands of years), difficulties related to partial lineage separation, and so forth (de Queiroz, 2005b, 2007). Importantly, these debates are empirical and methodological, not conceptual, and debates surrounding species boundaries will always be a part of the species problem (Sites and Marshall, 2004; Camargo and Sites, 2013). Species are hypotheses of lineage independence and are subject to empirical re-evaluation as new methods and data sets become available.

How does the complexity of the real world confound the diagnosis of evolutionary lineages? Figure 3A is adapted from a figure used by de Queiroz (1998, 1999, 2005b, 2007) to illustrate the relationship between lineage divergence and the evolution of the various contingent properties that
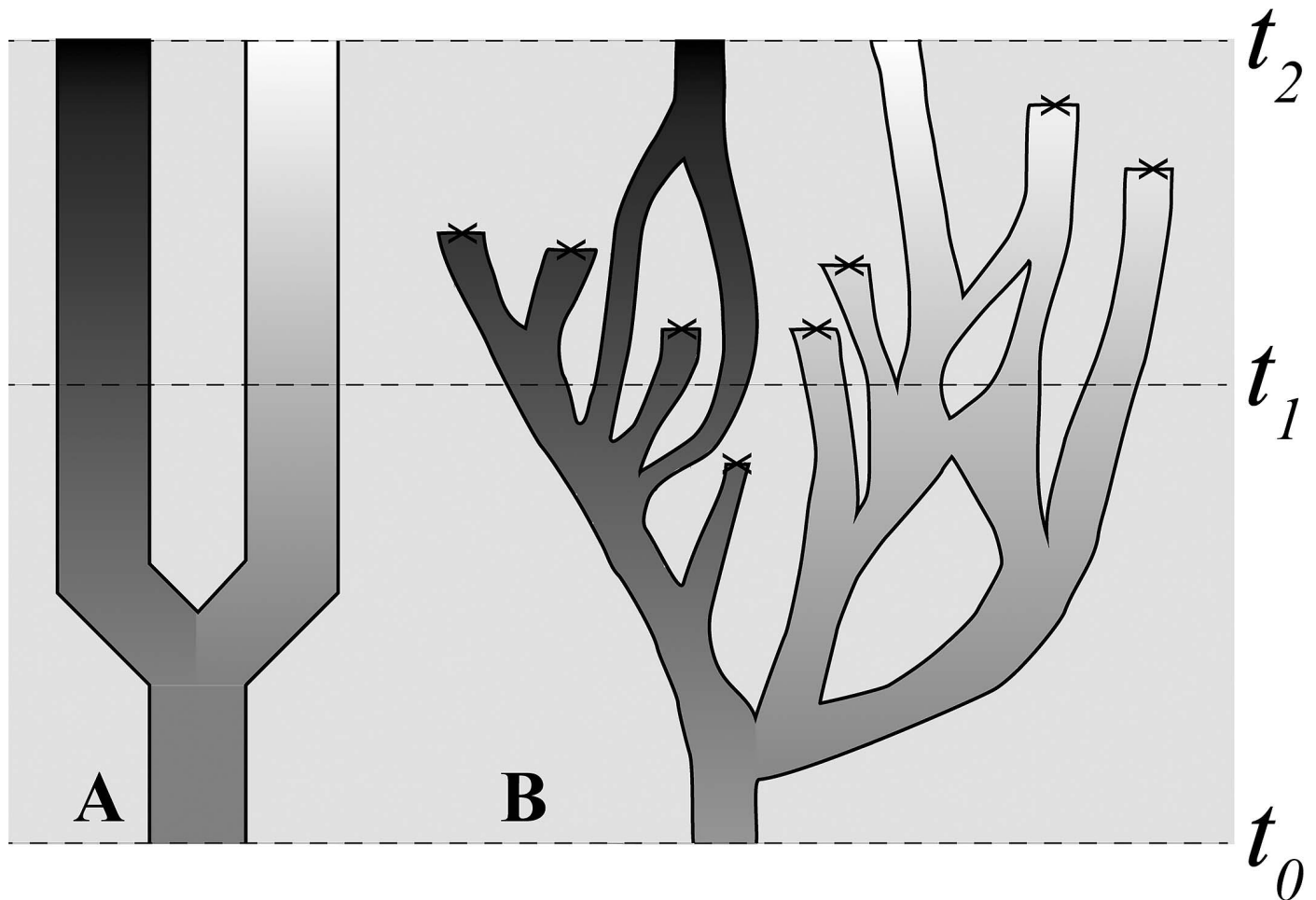
Fig. 3. (A) Diagram of lineage separation (species formation), modified from similar diagrams in de Queiroz (1998, 1999, 2005b, 2007). (B) Diagram of lineage separation in a complex that presents a complex array of lineages of differing degrees of evolutionary independence. Some time slices are straightforward to interpret. For example, time t_2 includes two species that would appear to a researcher to have arisen as in (A). But other time slices highlight the difficulties that arise as a consequence of the continuous nature of lineage separation. How many species should one recognize at time t_1? *Ensatina* presents an analogous situation.

underlie many species definitions. The figure is perfect for this purpose; however, we do wish to consider a less simplified alternative that may better reflect the process of species formation in some species complexes (Fig. 3B). Metapopulation-level lineages can be viewed as evolving like a braided river, with channels bifurcating and anastomosing over space and time. In this way, lineage independence is analogous to the evolution of reproductive isolation in that it is a continuum. When are two evolutionary lineages (or river channels) independent? Many species complexes (such as the *Ensatina* complex) present difficult situations in which the evidence for lineage divergence is ambiguous. Lineages may be partially separated, vary in their constituents over time, or be nested within other lineages (de Queiroz, 2005b, 2005c, 2007). This ambiguity is not a problem for evolutionary theory; it is an expected byproduct of gradual evolutionary change.

The USC does represent an important shift in thinking about some attributes of species, relative to most contemporary species definitions. One difference is that independent lineages are species even if young and undifferentiated: "Lineages do not have to be phenetically distinguishable, or diagnosable, or monophyletic, or reproductively isolated, or anything else, to be species. They only have to be evolving separately from other lineages" (de Queiroz, 2005c:205). If a pregnant lizard rafts out to an island and founds a new population, and that population is completely isolated from the mainland, is it a new species? It is under the USC, even if it has not yet diverged sufficiently from mainland populations to fulfill any of the contingent properties that are the focus of standard species definitions. In this sense, some species may be relatively cheap, or easily evolved. Will the USC lead to a huge proliferation of species? It could, but one can adhere to the USC and not advocate that all separately evolving lineages warrant taxonomic recognition (*c.f.*, Daugherty et al., 1990; Hay et al., 2010). Choosing which lineages to recognize taxonomically is analogous to choosing which population centers to recognize as cities on a map (O'Hara, 1993; de Queiroz, 1999, pers. comm.).

In an ideal ring species (Fig. 1A), terminal populations, though perfectly reproductively isolated, are connected by continuous intergradation around the ring. Despite sympatry among the terminal forms, this is a single species under both the USC and the BSC. The situation in real-world ring species is more complicated in that they typically consist of a mix of incompletely separated lineages, putative lineages that are diagnosed differently by different data sets, and lineages nested within lineages. In some instances, the number of species recognized within a ring species may be larger under the USC than under the BSC. But the taxonomic dimension, while important, is not the intended focus of the ring species concept. Ring species present, within a specific geographic

framework, an illustration of the evolution of a fundamental contingent property of species: reproductive isolation. Reproductive isolation is foundational for ecology and evolution because it inhibits reticulate evolution and because it leads to an increase in species richness within ecological communities by enabling sympatry (Coyne and Orr, 2004; Mallet, 2008b).

RING SPECIES, TAXONOMY, AND *ENSATINA*

The taxonomy of the *Ensatina* complex has received considerable attention, in print (Frost and Hillis, 1990; Graybeal, 1995; Highton, 1998) as well as in discussions at scientific meetings and in the anonymous feedback from grant and manuscript reviewers. Wake and colleagues have argued that the taxonomy of Stebbins (1949) be maintained while studies are ongoing. This approach has the advantage of maximizing stability, particularly as each new data set has conflicted with previous data sets in some way (Wake, 1997, 2006; Wake and Schneider, 1998). In addition, the taxonomy of Stebbins circumscribes ecologically relevant color pattern variants and draws attention to the ring species scenario. On the other hand, the taxonomy of *Ensatina* is formed around a contingent property of species (reproductive isolation), which may not correspond with the number of separately evolving evolutionary lineages. The number of independent evolutionary lineages, partial lineages, or even lineages within lineages within *Ensatina* is a thorny empirical issue and open to debate (Highton, 1998; Wake and Schneider, 1998; Wake, 2006; Pereira and Wake, 2009). Here is the key point: even if one concludes that multiple independent metapopulation-level evolutionary lineages (i.e., species) are present within the *Ensatina* complex, the complex would remain a ring species because it possesses the pertinent biogeographic history (Wake and Yanev, 1986; Moritz et al., 1992; Kuchta et al., 2009a) and patterns of reproductive isolation (Wake, 1997; Alexandrino et al., 2005; Pereira and Wake, 2009; Devitt et al., 2011). These attributes do not change whether *Ensatina* is composed of one independent metapopulation-level evolutionary lineage or 20 such lineages. It may be best to refer to *Ensatina* as a "ring complex" or a "ring species complex," to distinguish it from an idealized ring species composed of a single evolutionary lineage.

CONCLUSIONS

Ring species may be more common than realized but have been eliminated through taxonomic practice and by application of excessively strict criteria (Monahan et al., 2012). Alternatively, ring species may be rare because they require a specific biogeographic backdrop combined with a well-matched ecology (dispersal relative to geographic range), and because climatic shifts may cause them to exist only for a short period of time. Theoretical models suggest that ring species may be short lived, because even in the absence of shifting ranges and allopatry they are expected to fall apart into a complex of reproductively isolated units (Gavrilets, 2004). Whatever the case, ring species are important, not because they represent a mode of speciation, but because they are a premier illustration of the gradual (and usually messy; Fig. 3B) process of species formation. *Ensatina* salamanders, for example, exhibit geographic variation among populations, isolation by distance, divergent phenotypes, and secondary contacts characterized by admixture, tension zone dynamics, occasional hybridization, and com-

plete reproductive isolation. In *Ensatina*, the ring species scenario has been upheld in that (i) *Ensatina* evolved in the north and dispersed southward down two distributional axes; and (ii) at the southern terminus between these distribution arms, reproductive isolation is nearly complete, whereas elsewhere reproductive isolation is less advanced. The Greenish Warbler fulfills similar criteria in that it is a nearly complete ring characterized by a high level of reproductive isolation at one point in the ring. While Mayr considered ring species the "perfect demonstration of speciation" under the geographic speciation paradigm (Mayr, 1942, 1963), we have argued here that ring species are preeminent examples of Darwinian species formation. They exemplify how the same evolutionary processes that create patterns of geographic variation gradually accumulate in evolutionary lineages. By contrast, geographic speciation is focused on the necessity of geographic isolation for lineage differentiation (Mayr, 1942).

The ideal ring species is an unbroken chain of populations that evolved from a single ancestral population. There should be intergradation throughout, save for complete reproductive isolation at the point of terminal overlap, and the components of an ideal ring species should never have experienced even a single period of allopatry. Like all models, the ideal ring species is too simplified to describe any species complex accurately, and no ideal ring species are known. However, we side with Mayr in advocating for a focus on fundamental evolutionary processes—the interaction between biogeographic history and the evolution of reproductive isolating mechanisms—over a focus on strict criteria. *Ensatina* and the Greenish Warbler have both been characterized by periods of allopatry, have geographic breaks in their rings, and exhibit low levels of hybridization among terminal forms, yet they remain ring complexes. There has been much pressure in recent years to revamp the taxonomy of *Ensatina*. If this were done, we argue that *Ensatina* would remain a ring complex, because the biogeographic scenario and the patterns of reproductive isolation would remain intact. The requirement that ring species are necessarily linked to taxonomy is a throwback to the BSC, with reproductive isolation as a necessary property, as opposed to a contingent property, of species.

the years, including DEB-0317182 to SRK, and EF-0334939 and DEB-940834 to DBW.

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