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FURTHER OBSERVATIONS ON RELATIONSHIPS IN THE *ERYTHRANTHE GUTTATA* GROUP (PHRYMACEAE)

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ABSTRACT

Sequence order in the DIV1 inversion region is known for four taxa of the *Erythranthe guttata* group and provides basis for an inference of relationship. One sequence order is found in *E. grandis* and *E. guttata*, which are rhizomatous perennials; the reverse order is found in *E. microphylla* and *E. nasuta*, which are non-rhizomatous annuals. Because annuals are the prevalent life form in sect. *Simiola* as well as among species regarded as most closely related to sect. *Simiola*, and because of the simple developmental origin of rhizomes, annuals are reasonably hypothesized to be the primitive form in sect. *Simiola*, with rhizomatous species evolutionarily derived from annuals. The DIV1 sequence in *E. grandis* and *E. guttata* is thus the inverted one — these two species either arose from a common ancestor with the distinctive inversion or else one species is derived from the other. Each of five suppositions underlying this hypothesis of relationship is discussed: (1) rhizomes are a derived feature in sect. *Simiola*; (2) the DIV1 inversion has occurred only once; (3) *E. nasuta* is distinct from *E. grandis*-*guttata*-*microphylla*; (4) *E. grandis* is a distinct entity, separate from *E. guttata* and *E. microphylla*; and (5) *E. guttata* in the strict sense and *E. microphylla* are distinct entities. Reference to these entities as "ecotypes" within a single species underemphasizes their degree of evolutionary independence.

A chromosomal inversion has been discovered among four monkeyflower species of sect. *Simiola* (Lowry & Willis 2010) — *Erythranthe nasuta*, *E. microphylla*, *E. guttata*, and *E. grandis* (Lowry and Willis identified each of the latter three as an "ecotype" within a broadly considered *Mimulus guttatus*). This inversion region (DIV1) is located on chromosome 8 (Linkage Group 8). Remarkably, the inversion sequence is perfectly correlated with the life history features of the four species — one sequence occurs in *E. guttata* and *E. grandis*, which are perennial and rhizomatous, occur in habitats with year-round moisture, and flower relatively late in the season, while the opposite sequence occurs in *E. nasuta* and *E. microphylla*, which are annual and slender-taprooted or fibrous-rooted, occur in quickly drying habitats, and flower in early season. These critical differences are guided by major QTLs localized on the DIV1 inversion region. The inversion, with its tightly linked, locally adaptive alleles, contributes to significant isolating mechanisms between species with contrasting sequences. All plants of a single population have the same sequence, thus the inversion is not a "polymorphism," as characterized by Lowry and Willis, at least not in the traditional sense of that term.

Holeski et al. (2014) have confirmed the existence of the LG8 inversion and also have identified two others — one on chromosome 5 and another on chromosome 10. Little is yet known about the effects of loci within these latter regions.

Friedman and Willis (2013) subsequently discovered another broad feature controlled in part from LG8 that is consistently characteristic of *Erythranthe guttata* and *E. grandis*: both have a short-day dependent, cold-chilling, vernalization requirement, while annuals (*E. microphylla*, *E. nasuta*, *E. laciniata*, *E. pardalis*, and *E. nudata*) flower without vernalization. The difference in critical photoperiod is controlled by two, pleiotropic QTLs on LG8 — one of them appears to lie within the DIV1 inversion region. Differences in vernalization requirement are controlled by loci on LG 5, 6, 8, and 11 — Holeski et al. (2014) note that the LG5 marker resides within the chromosome 5 inversion identified in their study.

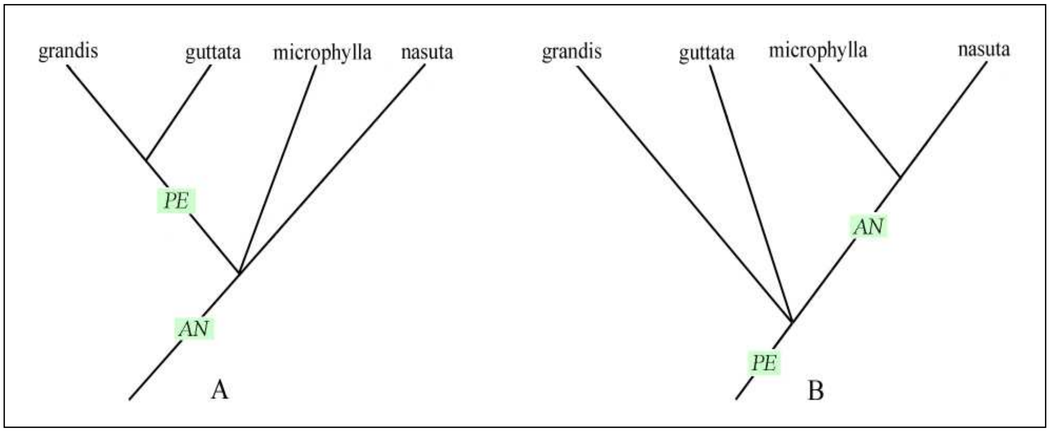
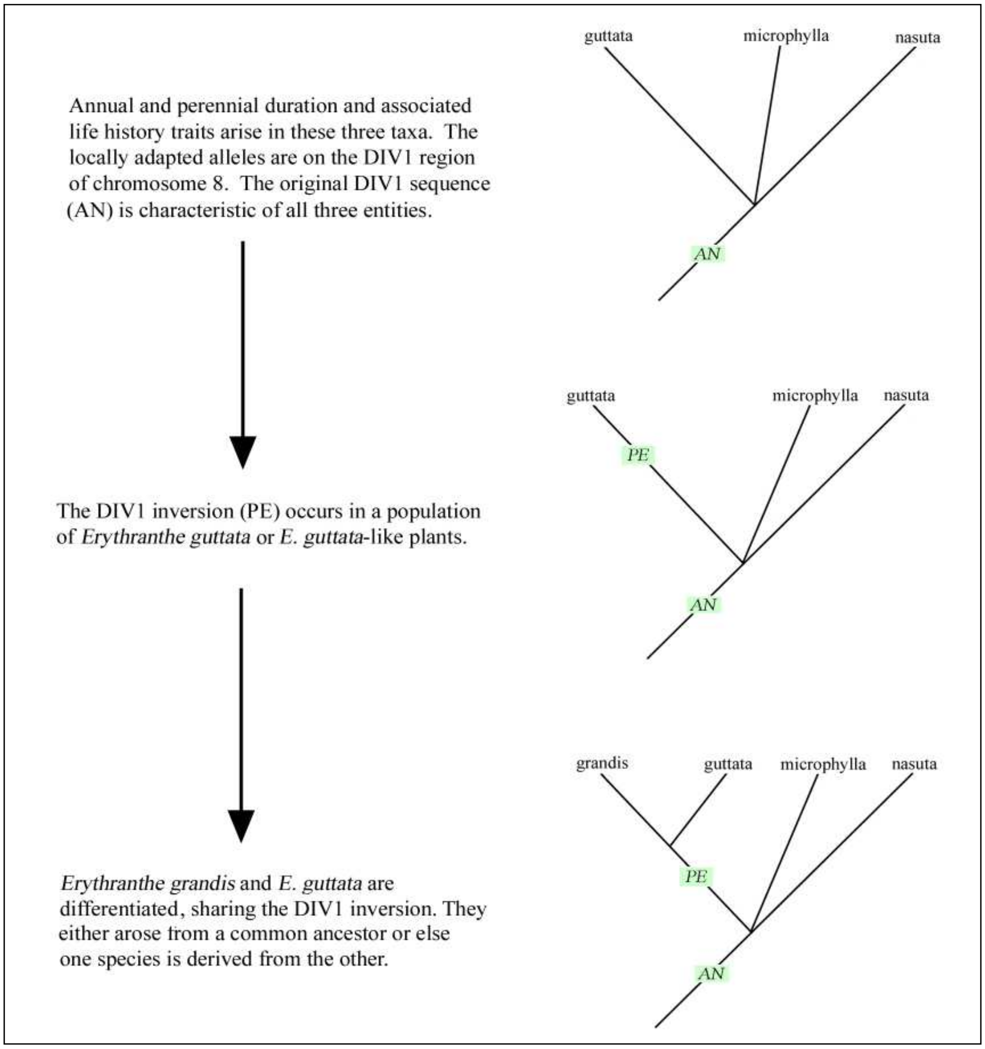


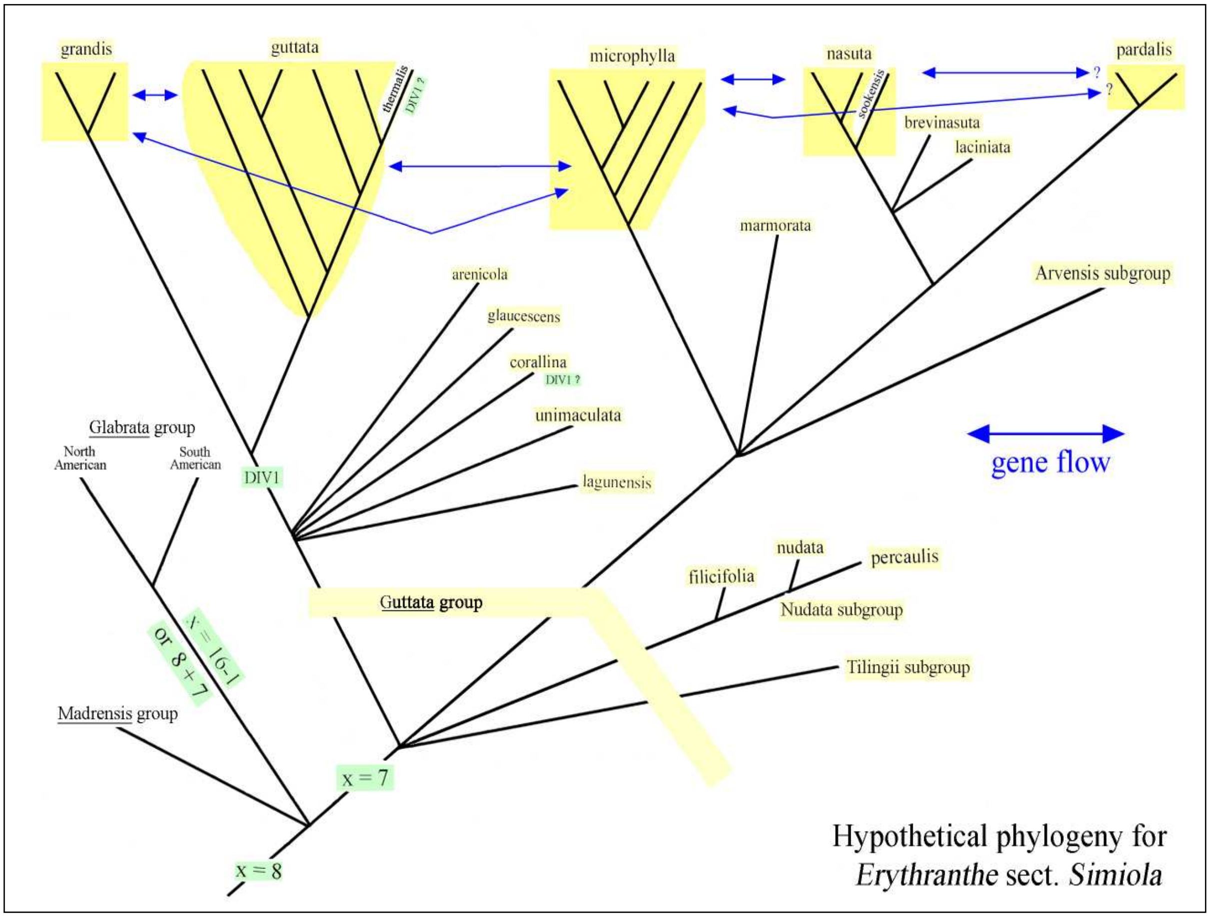
Figure 1. Cladistic distribution of the DIV1 inversion, as inferred from the sampling by Lowry & Willis (2010). A. If assumed that the DIV1 sequence in the annual taxa (AN) is the original state, that of the perennial taxa (PE) inverted, then *Erythranthe grandis* and *E. guttata* are sister taxa. B. If the perennial sequence were original, then *E. microphylla* and *E. nasuta* would be sisters.


Annual and perennial duration and associated life history traits arise in these three taxa. The locally adapted alleles are on the DIV1 region of chromosome 8. The original DIV1 sequence (AN) is characteristic of all three entities.

The DIV1 inversion (PE) occurs in a population of *Erythranthe guttata* or *E. guttata*-like plants.

Erythranthe grandis and *E. guttata* are differentiated, sharing the DIV1 inversion. They either arose from a common ancestor or else one species is derived from the other.

Figure 2. Origin and cladistic history of the DIV1 inversion, as hypothesized here for the four species studied by Lowry and Willis (2010).



Hypothetical phylogeny for *Erythranthe* sect. *Simiola*

Figure 3. Hypothetical phylogeny of *Erythranthe* sect. *Simiola* (modified from Nesom 2013). Taxa of the Guttata group are highlighted in light yellow. Extra branches within *E. grandis*, *E. guttata*, *E. microphylla*, *E. nasuta*, and *E. pardalis* indicate the existence of regional variants. *Erythranthe grandis* and *E. guttata* share the DIV1 inversion sequence of chromosome 8, here interpreted as a derived feature. Apart from species of the *E. tilingii* subgroup, *E. corallina* is the only other perennial, rhizomatous species of the Guttata group. *Erythranthe thermalis*, an annual, is derived directly from perennial *E. guttata* and would be expected to have the inverted sequence. The hypothesized 'microphylla-nasuta-pardalis' clade perhaps will prove to be without synapomorphic justification — it is depicted here as a clade to emphasize the observation that all included species are annual (but annual duration is hypothesized to be plesiomorphic in this group).

It is hypothesized here that annual duration (vs. perennial) is the primitive state in *Erythranthe*, with rhizomatous species evolutionarily derived from the annuals — the perennial DIV1 sequence is thus the inverted one. The derived features shared by *E. guttata* and *E. grandis* were either inherited from a recent common ancestor with the distinctive inversion or else one of the species is derived from the other (Fig. 1). A generalized series of events leading to the distribution of the inversion sequences is hypothesized in Figure 2. Evolutionary origin of at least some of the locally adapted alleles almost certainly preceded the occurrence of the inversion.

The hypothesis of relationship presented here makes five suppositions.

1. **Rhizomes are a derived feature in sect. *Simiola*.** The comments below are from Nesom (2014).

"Evolutionary change from perennial (rhizomatous) to annual and from annual to perennial apparently has occurred multiple times among species of *Erythranthe* (as well as in the Phrymaceae as a whole). It is suggested here that rhizomes and stolons in *E. guttata*, *E. corallina*, and *E. grandis* and in the *E. tilingii* group probably are derived features, arising from ancestors of annual duration.

All other x = 7 sect. *Simiola* species are annual, without rhizomes or stolons, as are most other species of *Erythranthe*. See related earlier comments (Nesom 2012)."

"Conceivably it is developmentally simple for lower branches to become rhizomelike or stolonlike by production of adventititous roots. Plants of *Erythranthe arvensis* and *E. cordata* characteristically are of annual duration (without rhizomes or stolons), but large plants in wet habitats sometimes become proximally decumbent or prostrate and develop adventitious roots at lower nodes and along the internodes. *Erythranthe glaucescens* is characteristically annual, but at least one rhizomatous population is known (Nesom 2012, p. 61; Taylor 2013) — the rhizomes (or runners) either arising independently from within the species or perhaps their genetic basis acquired by hybridization with *E. guttata*. Given the topology of Figure 1 [= Figure 3 in the present discussion], it is likely that the rhizomes of *E. tilingii*, *E. guttata*, and *E. corallina* are not strictly homologous but rather have arisen independently in each instance."

Species indicated at present to be evolutionary sister groups to sect. *Simiola* (sect. *Exigua*, with the single species *E. exigua*, and the sister pair sect. *Mimulosma* with 20 species and sect. *Mimulasia* with 11 species; Beardsley et al. 2004) mostly are annuals. Some species of sects. *Mimulosma* and *Mimulasia* are rhizomatous, but the same rationale applies to them — rhizomes probably are derivative.

2. **The DIV1 inversion has occurred only once**.

This is first an argument from parsimony. Origin of the DIV1 inversion through a single event is a simpler assumption than one of its independent occurrence in two otherwise very similar species. Biologically as well, independent occurrences and selection of an identical inversion are improbable.

3. ***Erythranthe nasuta* is distinct from *E. grandis-guttata-microphylla***.

In addition to its distinct morphology, ecology, and life history (e.g., Benedict et al. 2012; Nesom 2012), molecular-genetic studies also have shown *Erythranthe nasuta* to be distinct from *E. grandis*, *E. guttata*, and *E. microphylla* (e.g., Sweigart & Willis 2003; Sweigart et al. 2008; Oneal et al. 2014). In the Oneal et al. analysis, *E. nasuta* and *E. laciniata* are closely similar to each other and distinct from all the other taxa in markers from the inverted region of LG8; in the whole genome markers (outside of DIV1, K=4 through K=8), the two are distinct from each other and each also is unambiguously distinct from all other taxa.

Erythranthe nasuta and *E. microphylla* are broadly sympatric and natural hybrids between them are common. Introgression occurs between the two, though it is mostly unidirectional, into *E. microphylla* (Sweigart & Willis 2003; Martin & Willis 2007). Intrinsic postzygotic isolation exists (moderate F1 hybrid inviability with *E. microphylla* as seed parent) but is weak compared to prezygotic isolation — this, however, has not constrained the consistent recognition of *E. nasuta* as a distinct species (see related comments below regarding recognition of *E. grandis*, *E. guttata*, and *E. microphylla*).

4. ***Erythranthe grandis* is a distinct entity, separate from *E. guttata* and *E. microphylla***.

Erythranthe grandis is a distinct species (e.g., Nesom 2012, where it is analogous in concept to others, based on morphology and geography) and differences between it and *E. microphylla* in morphology, ecology, and genetics are documented in a series of studies comparing the "coastal perennial form of *Mimulus guttatus*" (= *E. grandis*) to the "inland annual form" (= *E. microphylla*) (Hall & Willis 2006; Lowry et al. 2008; Lowry et al. 2009; Hall et al. 2010; Lowry & Willis 2010). "Coastal perennial and inland annual populations of *M. guttatus* comprise two distinct morphologically and molecular genetically diverged groups. Nearly complete prezygotic isolation through a combination of geography, selection against immigrants, and flowering time isolation likely maintains the genetic differentiation of these coast and inland groups" (Lowry et al. 2008, p. 2209). Lowry et al. (2009) further found in reciprocal transplant studies that alleles of *E. grandis* at three salt spray tolerance loci are adaptive in the coastal perennial habitat but are not significantly disadvantageous in the inland annual habitat.

Erythranthe grandis also has been shown as genetically discrete within a sampling of sect. *Simiola* that included *E. guttata* (the "inland perennial"), *E. microphylla*, *E. nasuta*, *E. laciniata*, *E. nudata*, *E. tilingii*, and perhaps others. A neighbor-joining tree of these taxa based on *m*AP3 sequences shows a strongly supported cluster of five *E. grandis* samples from Marin Co. in central California to Lane Co. in central Oregon (Sweigart & Willis 2003, Group P in their Figure 3, 100% bootstrap support). Coastal perennials from California and Oregon also cluster together in the study by Oneal et al. (2014).

Lowry et al. (2008, p. 2211) noted that "Although our results demonstrate essentially complete prezygotic isolation and suggest that the coastal perennial and inland annual races of *M. guttatus* are in fact distinct biological species, the process by which most ecological races form, maintain their genetic distinctness, and accumulate further reproductive isolating barriers remains poorly understood." They were not explicit as to why they did not, in fact, regard *Erythranthe grandis* and *E. microphylla* as distinct species, but comments in their next paragraph (p. 2211) are closest to a statement in this whole series of studies regarding what further is necessary, in their opinion, for the speciation process to be completed. Apparently it is that "additional reproductive isolating alleles [must] spread between races" — i.e., "genic incompatibilities [that] are frequently involved in intrinsic postzygotic isolation" and which "facilitate the conversion of ecological races into good species." Thus in these monkeyflowers, following Lowry's review (2012) of stages in species formation, until the speciation process is 'irreversible,' they are considered ecotypes of a single species. One might have expected that discovery of the DIV1 inversion would be seen as a genic incompatibility contributing to irreversibility, but molecular-geneticists continue to regard "*Mimulus guttatus*" as comprising at least three "ecotypes."

In sum, *Erythranthe grandis* and *E. microphylla* are distinct in morphology, phenology, ecology, and genetic constitution, the two are nearly completely reproductively isolated (prezygotically), and a chromosome repatterning preserves the coherence of a suite of traits critical to their distinction. Reference to these entities as "ecotypes" of a single species underemphasizes their degree of evolutionary independence and does not account for their apparent cladistic history (they are not sister taxa nor does it seem probable that one is derived from the other). If the choice of terms is seen as essentially arbitrary (as reference to a particular point in a continuous speciation process), "species" is more accurate because what is meant by "ecotype" encompasses a much broader range of conditions and is correspondingly much more vague. Even if these entities have not achieved 'full' species status, as implied by Lowry and Willis (but which clearly is an arguable idea), they are much closer to that endpoint than to the beginning point of ecotypic differentiation.

The "inland perennial form of *Mimulus guttatus*" (= *Erythranthe guttata* in the strict sense) was not included in the series of molecular-genetic studies of *E. grandis*/*E. microphylla*, but compared to the annuals, plants of strict *E. guttata*, like *E. grandis*, also occur in a markedly different habitat (permanent water) and begin flowering considerably later. Recognition of reproductive isolation between *E. guttata* and *E. microphylla* completes a view of their relationship as analogous to that between *E. grandis* and *E. microphylla.*

Strict *Erythranthe guttata* and *E. grandis* are morphologically distinct between themselves and samples of inland perennial *E. guttata* were included in both Sweigart and Willis (2003) and Oneal et al. (2014), as noted above, where *E. grandis* formed a closely coherent cluster. In the hypothesis here, *E. guttata* and *E. grandis* are evolutionary sister taxa. Perhaps the most interesting experimental contrast within this small group of species remains to be done — between *E. guttata* and *E. grandis*.

The immediate ancestor of *Erythranthe grandis*/*guttata* may have been a population of *E. microphylla*-like plants, especially in view of the sympatry and demonstrated genetic overlap between *E. guttata* and *E. microphylla*. On the other hand, the annual *E. unimaculata* also is partially sympatric with *E. guttata* and otherwise bears a strong resemblance to it — it should reasonably be considered among possible progenitors. Morphological similarities of the annual *E. glaucescens* to *E. guttata* imply that an understanding of its evolutionary origin also may influence the interpretation of the relationship between *E. microphylla* and *E. guttata*. Similarly, study of *E. arenicola* may prove to have a direct bearing on such interpretations. And should the rhizomatous *E. corallina* prove to have the DIV1 inversion, such might imply that *E. microphylla* was even further removed from immediate ancestry of *E. grandis*/*guttata*.

Geographic variation exists within *Erythranthe grandis*. Plants from Oregon commonly produce nearly orbicular leaves and the stems tend to lie flat and produce adventitious roots along the prostrate portions. In the extreme, such plants are highly distinctive, even to the point that I once thought that they might deserve formal taxonomic recognition. Whether this form intergrades completely with the more guttata-like morphology (oblong leaves, erect stems) of southern *E. grandis* remains to be investigated.

5. ***Erythranthe guttata* in the strict sense and *E. microphylla* are distinct entities**.

With knowledge that the DIV1 inversion provides coherence for loci underlying a strong reproductive barrier between *Erythranthe grandis* and *E. microphylla*, it seems evident that a similar relationship exists between *E. guttata* and *E. microphylla*, which are distinct in morphology, habitat, phenology, and genetics. "Annual *M. guttatus* are nearly entirely distinct from perennial *M. guttatus* [including inland populations], although a few annual and perennial *M. guttatus* from California share some variation" (Oneal et al. 2014, p. 2581). Evidence perhaps exists for "limited introgression" in the inversion but most of the shared variation apparently is underlain by genetic similarities in the non-inversion regions, presumably reflecting the influence of gene flow, perhaps coupled with parallel selection. This shared, whole-genome variation presumably is what underlies the observation that "Published gene trees show *M. guttatus* to be a polytomy of annuals and perennials (Oneal et al. 2014, p. 2854).

Lowry and Willis (2010) described the geographic distribution of the DIV1 inversion as a mosaic of patchily distributed (corresponding to habit) annual and perennial populations of "*Mimulus guttatus*." The same concept is expressed by noting that *E. guttata* and *E. microphylla* occur as two species in close sympatry with distinctions maintained by reproductive isolation.

In the concept of current molecular-geneticists, "*Mimulus guttatus*" occurs both in annual and perennial ecotypes (e.g., Lowry et al. 2009; Lowry & Willis 2010; Oneal et al. 2014). In contrast, recognized species, across all genera and families, that are characterized by a mix of annual and perennial populations are rare or perhaps nonexistent (the rhizomatous population of *Erythranthe glaucescens*, noted above, is exceptional). Moyers and Rieseberg (2013) described populations of perennial plants as an ecotype within the otherwise annual species *Helianthus annuus* — these populations, however, are geographically localized, have other divergent characters (morphology, phenology, chemistry), and have been subsequently been formally recognized at specific rank (Stebbins et al. 2013). Some perennials may occasionally flower at a young age and thus might appear to be annual if observed only in that short moment of time, but this is not the case in monkeyflowers. Some perennial species may have rhizomatous and non-rhizomatous forms, but in the few such cases that I am aware of, there is a clear geographic distinction between the two forms. Among flowering plant species, annual and perennial duration generally appear to be regions of evolutionary stability, and the argument here removes a broadly considered "*Mimulus guttatus*" from consideration as a possible exception.

With reference to the *Erythrathe guttata* species group, Oneal et al. (2014, p. 2857) noted that "Our results suggest that even though considerable phenotypic differentiation exists between identifiable 'species,' there also exists **extensive shared neutral genetic variation across the complex** [emphasis added], which may ultimately undermine any clear taxonomy of the group. This common genetic variation is probably the result of shared ancestral polymorphism or ongoing gene flow, which has occurred despite high levels of reproductive isolation ... between members of the complex." This is perhaps not a particularly remarkable observation, because in common parlance the "extensive shared neutral genetic variation across the complex" surely mirrors the idea that they are members of the same genus (i.e., that the "shared neutral genetic variation" indeed is "shared ancestral polymorphism"). Such probably is the case in many genera, partcularly those that are relatively recently evolved and characterized by many species separated by small differences.

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