Incongruence Between Morphological and Mitochondrial-DNA Characters Suggests Hybrid Origins of Parthenogenetic Weevil Lineages (Genus *Aramigus*)

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Abstract.—An expanded matrix of morphological characters for the genus *Aramigus* (Coleoptera: Curculionidae), which includes numerous polyploid parthenogenetic lineages, was compared and combined with a published matrix of mitochondrial DNA (mtDNA) characters. The matrix of morphological characters provides little resolution of the *A. tessellatus* and *A. uruguayensis* species complexes but does resolve previously unresolved relationships among other morphologically defined species (*A. globoculus*, *A. intermedius*, *A. curtulus*, *A. planioculus*). The morphological and mtDNA characters are significantly incongruent (*I* = 0.435, *I* = 0.463; *I* = 0.0735), according to the tests of Farris et al. (*P* = 0.010) and Templeton (*P* < 0.005), probably because of hybrid origins of polyploid parthenogenetic lineages. For the few sexual lineages included in both matrices, morphology and mtDNA provide congruent estimates of phylogeny. In spite of recent injunctions against combining data sets that are incongruent because of differing histories, the results of the combined analyses were used to select one of the most-parsimonious mtDNA trees as the best estimate of maternal-lineage genealogy and to reconstruct the evolution of parthenogenesis under the assumption that transitions from sexuality to parthenogenesis are irreversible. Where cytogenetically justified, as in weevils, the irreversibility assumption is useful for producing conservative estimates of the age of parthenogenetic lineages in spite of potential sampling bias against sexuals. [Ancient asexuals; Entiminae; hybridization; incongruence; Naupactini; parthenogenesis; reticulation; root weevils.]

The rise of molecular systematics has been accompanied by a persistent controversy over how to interpret conflicts between morphological and molecular characters (Paterson, 1987; Swofford, 1991). One response to the problem has been the invention and growing use of statistical tests of character incongruence (Templeton, 1983; Larson, 1994; Farris et al., 1995; Poe, 1996), which often fail to reject the null hypothesis of homogeneity even when the trees from the separate analyses differ greatly (Sites et al., 1996). In some cases, however, the incongruence between morphological and molecular estimates has been found to be significant (Miyamoto, 1996; Poe, 1996; Engel and Schultz, 1997). Explanations for such incongruence fall into two classes: (1) error in phylogenetic inference for one or both data sets, and (2) a difference in phylogenetic history between the two data sets (de Queiroz et al., 1995). Error in phylogenetic inference is thought to result from convergence, particularly from convergences caused by very different rates of evolution between taxa (Sytsma, 1990; Kadereit, 1994). Differences in phylogenetic history have been thought to result from errors in orthology assessment, from lineage sorting, or from reticulation (Sytsma, 1990; Soltis and Kuzoff, 1995; Birky, 1996).

In plants, reticulation has widely been thought to be important (McDade, 1995). Incongruence between morphological and molecular characters in plant taxa has often been interpreted as having resulted from a history of reticulation (Sytsma, 1990; Soltis and Kuzoff, 1995; Schilling and Panero, 1996). In contrast, in animals, cases of incongruence are more often ascribed to some sort of error (Miyamoto, 1996; Poe, 1996; Engel and Schultz, 1997). Parthenogenetic animal lineages represent a peculiar case. In vertebrates, almost every parthenogenetic lineage has now been shown to be of hybrid origin (Dawley and Bogart, 1989; Cole and Dessauer, 1993; Cole et al., 1993; Moritz, 1993; Cole et al., 1995; Radtkey et al., 1995). In parthenogenetic line-
ages in other animal taxa, the importance of hybrid origins is far less clear, though the number of examples is growing (Dowling and Secor, 1997; Giessler, 1997; Parker and Niklasson, in press). For no parthenogenetic animals have molecular and morphological data sets been directly compared to look for patterns of incongruence. Here, we present a case of strong incongruence between morphological and mtDNA data sets for a genus of weevils (Aramigus) that includes a large number of parthenogenetic lineages, and we interpret this incongruence as likely to be the result of hybrid origins of parthenogenesis. It has been argued that when data sets are incongruent, and particularly when that incongruence is thought to reflect a difference in phylogenetic history, the sets should not be combined (de Queiroz et al., 1995). However, we do perform combined analysis of our data, which we argue helps us to choose the best-supported estimate of maternal-lineage genealogy among the most-parsimonious mtDNA trees.

The evolution of parthenogenetic lineages is of great theoretical interest to evolutionists because it bears directly on a major unsolved problem, the adaptive significance of sexuality. A basic question is whether any parthenogenetic lineages have survived for very long periods, or whether all are of recent origin (Maynard Smith, 1992; Judson and Normark, 1996). Available phylogenetic data on this question have been subject to sharply different interpretations. We discuss the basis of this controversy, which hinges in part on the problem of adequate taxonomic sampling and in particular on the potential in some groups (such as Aramigus) for systematic underrepresentation of sexual lineages. To minimize the effects of such bias and to produce conservative estimates of clonal antiquity, we invoke an assumption of irreversibility for transitions from sexuality to parthenogenesis, which we argue is reasonable, given the cytogenetic details of sexuality and parthenogenesis in weevils.

**Materials and Methods**

**Sources of Data**

The mtDNA data are from Normark (1996a). The matrix of morphological characters is based on that of Lanteri and Díaz (1994), but has been expanded and modified as described below. The single life-history character that we discuss, sexuality vs. parthenogenesis, was not included in the phylogenetic analysis.

**Morphological Characters**

**Taxonomic units.** — In addition to the out-group, there are 19 terminal taxonomic units within the genus Aramigus that are distinguishable by morphological characters (Table I): six species outside of A. tessellatus that have been treated as morphologically uniform (A. pilosus Lanteri, A. curtulus (Hustache), A. planioculus Lanteri, A. globoculus Lanteri, A. intermedius Lanteri, A. conirostris (Hustache)), two lineages of A. uruguayensis Normark and Lanteri, three morphotypes of A. tessellatus that have been treated as morphologically uniform (biseriatus (Hustache), durius (Germar), and tessellatus (Say)), two color variants of the viridipallens (Hustache) morphotype, two color variants of the santafecinus (Lanteri) morphotype, and four variants of the pallidus Horn morphotype. Three of these four variants have been described as pallidus forms 1, 2, and 3 (Lanteri et al., 1987; Lanteri and Díaz, 1994), and are here called pallidus I, pallidus II, and pallidus III. The fourth variant has a curled spermathecal duct, as illustrated by Lanteri and Díaz (1994; their Fig. 59), and is here called pallidus IV. Aramigus uruguayensis was treated as the basal “uruguay” morphotype of A. tessellatus by Normark (1996a) but has since been recognized as a separate species complex (Normark and Lanteri, 1996).

**Reevaluation of characters.** — Characters were taken from Lanteri and Díaz (1994) with a few modifications. Five characters that serve to distinguish morphological variants (morphotypes) of A. tessellatus have been added to the matrix: characters 16 (elytral width), 19 (apex of sternum 5), 22 (vestiture color), 25 (ramus of spermatheca), and 26 (length of cornu of spermatheca). Thus the total number of morphological characters is now 32, of which 19 are cladistically informative for morphologically defined taxa. States have been added or deleted for several characters (2, 7, 21, 29, 30). The new matrix of
morphological characters is given in Appendices 1 and 2.

### The Combined Matrix

In the mtDNA data set, taken from Normark (1996a), there are 33 terminal taxa. Terminal taxa are not identical between the two data sets because (1) mtDNA data are missing for five taxa (three basal species \(A. pilosus, A. curtulus\), and the undescribed outgroup) and two morphotypes of \(A. tessellatus\) [biseriatus, pallidus I]; (2) some morphologically defined taxa are represented by multiple mtDNA haplotypes; and (3) in one case, two morphologically defined taxa (pallidus II and pallidus III) share a single mtDNA haplotype (pallidus B3). (Throughout this paper, names of species and morphotypes are given in italics; names of haplotypes and haplotype groups in roman type.) When all the data are combined into a single matrix, there are 39 taxa (of which 5 lack mtDNA data) and 794 characters (762 base pairs \([bp]\) of mtDNA + 32 morphological characters), of which 208 (186 \([bp]\) + 22 morphological characters) are informative. If the 5 taxa

<table>
<thead>
<tr>
<th>Designation</th>
<th>Species</th>
<th>Morphotype</th>
<th>Haplotype</th>
<th>Reproduction(^a)</th>
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<td>?</td>
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<td>?</td>
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<td>?</td>
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<td>uruguayensis B1</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>uruguayensis B2</td>
<td>(A. uruguayensis)</td>
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<td>?</td>
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</tr>
<tr>
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<td>durius</td>
<td>durius 2</td>
<td>P</td>
</tr>
<tr>
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<td>tessellatus 1</td>
<td>P</td>
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<tr>
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<td>santafecinus brown</td>
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<td>santafecinus br A2</td>
<td>(A. tessellatus)</td>
<td>santafecinus brown</td>
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<td>santafecinus brown</td>
<td>santafecinus B</td>
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</tr>
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<td>santafecinus gy C1</td>
<td>(A. tessellatus)</td>
<td>santafecinus gray</td>
<td>santafecinus C1</td>
<td>P</td>
</tr>
<tr>
<td>santafecinus gy C2</td>
<td>(A. tessellatus)</td>
<td>santafecinus gray</td>
<td>santafecinus C2</td>
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<td>(A. tessellatus)</td>
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<td>santafecinus C3</td>
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<td>(A. tessellatus)</td>
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<td>(A. tessellatus)</td>
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<tr>
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<tr>
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<td>viridipallens brown</td>
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<td>P</td>
</tr>
<tr>
<td>viridipallens br B3</td>
<td>(A. tessellatus)</td>
<td>viridipallens brown</td>
<td>viridipallens B3</td>
<td>P</td>
</tr>
<tr>
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<td>?</td>
</tr>
<tr>
<td>pallidus II A1</td>
<td>(A. tessellatus)</td>
<td>pallidus II</td>
<td>pallidus A1</td>
<td>S</td>
</tr>
<tr>
<td>pallidus II A2</td>
<td>(A. tessellatus)</td>
<td>pallidus II</td>
<td>pallidus A2</td>
<td>S</td>
</tr>
<tr>
<td>pallidus II B1</td>
<td>(A. tessellatus)</td>
<td>pallidus II</td>
<td>pallidus B1</td>
<td>P</td>
</tr>
<tr>
<td>pallidus II B2</td>
<td>(A. tessellatus)</td>
<td>pallidus II</td>
<td>pallidus B2</td>
<td>P</td>
</tr>
<tr>
<td>pallidus II B3</td>
<td>(A. tessellatus)</td>
<td>pallidus II</td>
<td>pallidus B3</td>
<td>P</td>
</tr>
<tr>
<td>pallidus III B3</td>
<td>(A. tessellatus)</td>
<td>pallidus II</td>
<td>pallidus B3</td>
<td>P</td>
</tr>
<tr>
<td>pallidus IV C</td>
<td>(A. tessellatus)</td>
<td>pallidus IV</td>
<td>pallidus C</td>
<td>?</td>
</tr>
<tr>
<td>biseriatus</td>
<td>(A. tessellatus)</td>
<td>biseriatus</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

\(^a\)S = sexual; P = parthenogenetic.
lacking mtDNA are excluded, 34 taxa and 792 characters remain, though of these only 21 morphological characters are variable and 18 are informative, for a total of 204 informative characters. MacClade 3.04 (Maddison and Maddison, 1993) was used for compiling and editing the data matrix.

Choice of Outgroup

Aramigus is part of the large (~500 spp.) and taxonomically problematic Naupactus–Pantomorus complex (Lanteri and Normark, 1995). The genus is defined mostly by characters of its highly unusual spermatheca and sclerotized spermathecal duct. In most other characters it is very similar to the other species in the complex that have reduced humeri and were historically placed in the polyphyletic genus Pantomorus. The undescribed species here used as the outgroup is thought to be very close to Aramigus on the basis of its strongly conical rostrum, very convex eyes, coarse elytral setae, and overall similarity in the shape of pronotum and elytra. The position of A. planioculus as the most basal of the species sampled for mtDNA is supported by five characters (15, 16, 22, 30, 31), reflecting its short, narrow spermathecal duct; short, wide elytra; and yellow vestiture.

Separate Analyses of mtDNA and Morphology

mtDNA Analysis 1.—We calculated the most-parsimonious trees (MPTs) from the mtDNA data alone for the 34 taxa for which they were available, using a heuristic search with 100 random-addition-sequence starting trees (RSTs) in PAUP 3.1.1 (Swofford, 1993). We estimated bootstrap values by using 100 replicates (with 10 RSTs per bootstrap replicate), and estimated branch support (Bremer, 1994) as follows: Branch supports (decay indices) of 1 and 2 were identified by finding (using 100 RSTs) all trees 1 to 2 steps longer than the MPTs and constructing strict consensus trees to see which branches collapsed relative to the MPTs; higher branch supports were estimated by constructing a constraint expressing each of the remaining internal branches and finding the length of the MPTs that were not compatible with the constraint. (Heuristic searches of constrained trees sometimes used as few as 10 RSTs; in cases where any of the first 10 replicates failed to find the same MPTs, the analysis was continued for a total of 100 RSTs.)

mtDNA Analysis 2.—We excluded all taxa from which males are unknown and calculated MPTs by exhaustive search for the six “taxa” known to represent sexual populations (i.e., the haplotypes globoculus, intermedius B1, intermedius B2, uruguayensis A, pallidus A1, and pallidus A2). Unlike most haplotypes in this study, intermedius B1 and B2 cannot be thought of as representing independent organismal lineages, because they are known to have been drawn from the same sexual population. The same is true of pallidus A1 and A2. They, and the suites of morphological characters corresponding to them, are formally treated here as “taxa” for ease of comparison with other analyses in this study.

Morphology Analysis 1.—We estimated MPTs by heuristic search (with 100 RSTs), using only the morphological data for all 39 taxa. We estimated branch supports as for mtDNA Analysis 1.

Morphology Analysis 2.—As Morphology Analysis 1, but without morphotype pallidus 1.

Morphology Analysis 3.—For ease of comparison with the mtDNA results, we estimated MPTs by heuristic search (with 100 RSTs), using only the morphological data for the 34 taxa used in mtDNA Analysis 1.

Morphology Analysis 4.—For ease of comparison with the results of Lanteri and Díaz (1994), we calculated MPTs by branch-and-bound, using only the morphological characters for the 20 morphologically distinguishable taxa.

Morphology Analysis 5.—As Morphology Analysis 4, but without morphotype pallidus 1.

Morphology Analysis 6.—We calculated MPTs by exhaustive search, using only the morphological data for the six “taxa” known to be sexual. These represent only four morphologically distinguishable taxa. For each analysis, after finding MPTs for equal character weights (EW), we applied successive weighting (SW) (Farris, 1969), as imple-
Incongruence between Data Sets

We calculated the range of values for Miyamoto’s incongruence index $I_M$, as described by Swofford (1991), across the EW MPTs for Morphology Analysis 3 and mtDNA Analysis 1 (i.e., the analyses that include all 34 taxa for which mtDNA data are available, and no others). Using a pair of trees for which $I_M$ was minimal, we performed Templeton’s (1983) nonparametric test of the fit of the two sets of characters to the alternate tree topologies, according to the method described in Larson (1994). From the combined matrix, we also calculated the Mickevich and Farris (1981) incongruence index $I_{MF}$ and tested the significance of this incongruence according to the method described in Farris et al. (1995), using the program xarn (Farris, 1996).

Consensus Analyses

We calculated four strict consensus trees:

1. EW MPTs from mtDNA Analysis 1 and Morphology Analysis 3.
2. SW trees from mtDNA Analysis 1 and Morphology Analysis 3.
3. EW MPTs from mtDNA Analysis 1 and pruned (to remove the five taxa missing mtDNA characters) EW MPTs from Morphology Analysis 1.
4. SW trees from mtDNA Analysis 1 and pruned SW trees from Morphology Analysis 1.

Combined Analysis

Combined Analysis 1.— We searched (using 100 RSTs) for MPTs for the entire matrix, with all taxa and all available characters included.

Combined Analysis 2.— We excluded only the morphotype pallidus I and searched for MPTs as above.

Combined Analysis 3.— We excluded the five taxa missing mtDNA characters and searched for MPTs as above.

Evolution of Parthenogenesis

Using MacClade, we optimized the evolution of parthenogenesis on the EW MPTs of Combined Analysis 2, both with and without the assumption that transitions from sexuality to parthenogenesis were irreversible.

Results

Separate Analyses of mtDNA and Morphology

mtDNA Analysis 1.— Results under EW were essentially identical to those of Normark (1996a), differing only by the presence of two terminal taxa having the pallidus B3 haplotype. Hence there were 14 MPTs (length = 498, consistency index [CI] = 0.564, retention index [RI] = 0.785), whose strict consensus is essentially the same as that shown in Figure 2 of Normark (1996a). SW produced a single, slightly different tree (length = 499), shown in the right side of Figure 1.

mtDNA Analysis 2.— A single MPT was found under EW (length = 157, CI = 0.917, RI = 0.892), with unrooted topology identical to the topology for these taxa in Figure 1 (globoculus (intermedius B1, B2) uruguayensis A (pallidus A1, A2)), unchanged under SW. The primary internal branch, separating globoculus and intermedius B from uruguayensis A and pallidus A, has a branch support of 24.

Morphology Analyses 1–5.— All MPTs resulting from the morphology analyses are consistent with the topology (A. pilosus (A. curtulus, A. planiocular) ((A. globoculus, A. intermedius) (A. conirostris, A. tessellatus))) that is shown in Figure 74 of Lanteri and Díaz (1994) and reflected in the left side of Figure 1 here. The MPTs from the different analyses differ with respect to the resolution and topology of A. conirostris, A. uruguayensis, and the morphotypes of A. tessellatus. In the strict consensus trees for the EW analyses, there is always a major polytomy for A. uruguayensis and the morphotypes of A. tessellatus. (In Morphology Analysis 3 under EW, A. conirostris is included in this polytomy; all other analyses resolve it as the sister-species of A. uruguayensis + A. tessellatus.) In analyses in which single morphotypes are multiply repre-
sented (Morphology Analyses 1–3), only durius and viridipallens consistently appear as monophyletic; pallidus, santafecinus, and their various sub-forms never do. The only other point of resolution within this polytomy under EW is a sister-group relationship between the durius and viridipallens morphotypes, which appears in the two analyses (Morphology Analyses 2 and 5) from which only pallidus I has been omitted.

There is much more resolution in the SW analyses. A strict consensus of two SW trees from Morphology Analysis 2 is shown in the left side of Figure 1. SW trees from other analyses show much more resolution. A comparison of trees produced by using the morphological and molecular data from Aramigus. The morphology tree is a strict consensus of two successive-weighting (SW) trees from the analysis of 32 morphological characters. The set of MPTs under equal weights (EW) included these two trees and one additional tree. Length = 65; CI = 0.738; RI = 0.883. Numbers over the branches indicate branch support (Bremer, 1994) under EW. Branches with support 0 are those that do not appear in the third EW tree. The mtDNA tree is the SW tree for the 762 mtDNA characters. Length under SW = 499, one step longer than the MPTs under EW. Numbers over the branches again indicate branch support; the branch with support – 1 is the one that does not appear in the MPTs under EW; those with support 0 appear in some but not all MPTs under EW. Numbers below the branches indicate bootstrap percentages. The box encloses the names of taxa that are within the A. tessellatus complex. Names of terminal taxa have up to 4 parts (see Table 1), in the following order: (1) the name of the species (outside box) or morphotype (inside box) (Lanteri and Díaz, 1994); (2) in the case of some polytypic morphotypes, an indication of the variant form of the morphotype, i.e., for the pallidus morphotype, a roman numeral referring to the form of the morphotype, or for the santafecinus and viridipallens morphotypes, an abbreviation indicating the color variant (br = brown; gy = gray; gn = green); (4) for species and morphotypes represented by more than one mtDNA haplotype group, a capital letter specifying the haplotype group (Normark, 1996a); 5) in the case of haplotype groups represented by more than one haplotype, an Arabic numeral referring to the haplotype.
yses lacking pallidus I (Morphology Analyses 3 and 5) have topologies consistent with this. Analyses that include pallidus I (Morphology Analyses 1 and 4) yielded SW trees that resolve A. uruguayensis and the A. tessellatus complex quite differently (durius (biseriatus (tessellatus ((sexual uruguayensis, parthenogenetic uruguayensis, pallidus IV) pallidus I (pallidus II (pallidus III, gray santafecinus (brown santafecinus (viridipallens))))))).

Morphology Analysis 6.—A single MPT was found under EW (length 17, CI 1.0, RI 1.0), identical to the MPT of mtDNA Analysis 2, unchanged by SW, with branch support 6 for the internal branch.

Incongruence

$I_M$ ranges from 0.435 to 0.463, and $I_{MF}$ is 0.0735. The morphological and mtDNA characters are significantly incongruent, according to the test of Farris et al. (1995 ($P = 0.010$)). The most-congruent morphology and mtDNA trees (from Morphology Analysis 3 and mtDNA Analysis 1) differ significantly in their fit to the two data sets, according to Templeton's test ($P < 0.005$ for each).

Consensus of Separate Analyses

There is little resolution in any of the four consensus trees, which each have three or four resolved nodes. Of these, two or three nodes simply resolve single species (A. conirostris) or morphotypes (durius and sometimes tessellatus). The remaining node, appearing in consensus trees 2, 3, and 4, but not consensus tree 1, is A. uruguayensis + A. tessellatus.

Combined Analysis

Figure 2 shows one of the 3 EW MPTs (the single SW tree) of Combined Analysis 2 (length = 580, CI = 0.567, RI = 0.782). The strict consensus trees for all the other combined analyses are consistent with this one and are less resolved, except for the SW tree (length = 561) for Combined Analysis 3, which shows (like the morphology trees) A. conirostris as the sister species of A. uruguayensis + A. tessellatus. Under EW, Combined Analysis 3 resulted in 7 MPTs (length = 560, CI = 0.562, RI = 0.781); the intersection of this set of trees and the set of EW MPTs from mtDNA Analysis 1 contains three trees. One of these conflicts with Figure 2 only in the placement of A. conirostris (see Fig. 1, right side); the other two also differ in the position of viridipallens A.

Evolution of Parthenogenesis

The evolution of parthenogenesis on one of the combined-analysis trees (Fig. 2) is shown with and without the assumption of irreversibility in Figures 3 and 4, respectively. Under irreversibility (Fig. 3), there are a minimum of six origins of parthenogenesis in the genus and possibly as many as seven other origins, depending on the reproductive states (parthenogenetic vs. sexual) of the six lineages shown for which the reproductive state is not known, and on the placement and reproductive state of pallidus I. For the other 2 EW MPTs for Combined Analysis 2, an additional origin of parthenogenesis is required. When irreversibility is not assumed (Fig. 4), there is a single, early transition from sexuality to parthenogenesis with three subsequent reversions to sexuality.

Discussion

Incongruence

Morphological and mtDNA characters for the genus Aramigus are strongly incongruent with each other. This is seen most clearly in the result of the test of Farris et al. (1995). The primary criticism of the metric on which this test is based has been that it is too conservative, and understates incongruence (Swofford, 1991), but in this case the incongruence is found to be highly significant. This evidence suggests that morphological and mtDNA characters in Aramigus have had different evolutionary histories, which supports other indirect evidence that parthenogenetic lineages of Aramigus, and weevils in general, are of hybrid origin.

In the A. tessellatus complex, hybrid origins of parthenogenetic lineages are suggested by the distribution of ploidy levels (Normark, 1996b); most lineages are triploid and a few have higher levels of polyploidy. This pattern is similar to that seen in other parthenogenetic broad-nosed weevils, and has been interpreted as being the result of successive additions of haploid genomes through hybridization with.
FIGURE 2. The single SW tree (one of 3 EW MPTs) for Combined Analysis 2. Length = 580, CI = 0.567, RI = 0.782. 

p = the pallidus I morphotype joins to that branch in some MPTs of Combined Analysis 1 under EW. The first two numbers above a branch indicate branch support under Combined Analyses 1 and 2, respectively. A third number indicates branch support under Combined Analysis 3; when underlined, it indicates support for that branch exclusive of the biseriatus morphotype (which was not included in Combined Analysis 3). We interpret this tree as our best estimate of maternal-lineage genealogy in Aramigus.
sexual lineages (Saura et al., 1993). Note that this pattern of polyploidization should lead to particularly strong incongruence between mitochondrial and morphological characters, under the assumptions that mitochondrial inheritance is primarily maternal and that morphology is primarily encoded by nuclear genes, because only one of the haploid nuclear genomes is contributed by an egg, whereas two or more are contributed by sperm. Support for hybrid origins of other parthenogenetic weevil lineages comes from a reinterpretation of allozyme data on Otiorhynchus scaber, which found support for multiple origins of parthenogenesis through hybridization between the sampled sexual population and one that has not been sampled (and that may be extinct) (Tomiuk and Loeschcke, 1992).
The lineages of *Aramigus* that are known to be sexual show complete taxonomic congruence of mitochondrial and morphological characters, with high branch support (mtDNA Analysis 2 and Morphology Analysis 6). This supports the hypothesis that the incongruence is due to the parthenogenetic lineages; however, this support is not strong, because these sexual lineages are few and are well separated phylogenetically.

**Reanalysis of Morphology**

The purely morphological analysis presented here differs somewhat from the results presented by Lanteri and Díaz (1994). This is presumably due to the division of *A. tessellatus* into several morphotypes, the addition of the new species *A. uruguayensis*, the addition of five characters, and the reevaluation of several other characters (Appendix 1). One of the new
characters, vestiture color, was added specifically because it appeared to correlate with mtDNA haplotype (Normark, 1996a). All of the morphological analyses in this study resolve two nodes that Lanteri and Díaz’ study left unresolved: (A. curtulus + A. planio-culus) and (A. globoculus + A. intermedius).

Our decision to delete pallidus I from some analyses (Morphology Analyses 2 and 4 and Combined Analysis 2) was because of its very unstable position in the analyses that included it. The unstable position of pallidus I and low branch supports in an analysis including it (Combined Analysis 1) are indicated in Figure 2. Analyses of morphology that excluded pallidus I were stable to successive weighting; those including it were not.

Conflict and Agreement between mtDNA and Morphology

Despite the incongruence between the mtDNA and morphological data sets, there are some points of agreement. Under EW, both data sets yield a major polytomy at the base of the A. tessellatus complex. The morphotypes of A. tessellatus tend to have the same phylogenetic status in both the mtDNA and morphology MPTs, in the following sense. All the relevant analyses (mtDNA Analysis 1 and Morphology Analyses 1–3) show monophyly for durius, and all of these but Morphology Analysis 1 show monophyly for the tessellatus morphotype. The status of pallidus is unresolved in all of these analyses under EW, though all the MPTs from the combined analyses that exclude pallidus I (Combined Analyses 2, 3) show a monophyletic pallidus (Fig. 2). (SW trees of mtDNA Analysis 1 and Morphology Analyses 2 and 3 resolve pallidus as nonmonophyletic [Fig. 1].) The santafecinus morphotype is nonmonophyletic in the mtDNA trees, unresolved in the EW morphology trees, and nonmonophyletic in the SW morphology trees. The one morphotype on whose status the data sets disagree is viridipallens, which is monophyletic according to morphology and nonmonophyletic according to mtDNA.

Several points of disagreement between the trees result from the resolution by mtDNA of morphologically-defined taxa into nonmonophyletic groups of distinct lineages, i.e., intermedius A and B, santafecinus A, B, and C, and viridipallens A and B. In the case of A. intermedius, the two mtDNA lineages intermedius A and intermedius B have highly (11%) divergent mtDNA haplotypes. The sample from which the intermedius B haplotypes were obtained consisted of three males, whereas the intermedius A haplotypes came from a sample of two females; thus, genital and other sex-specific characters could not be directly compared between the two lineages. It seems likely that morphological differences will yet be found to distinguish between these two lineages, which appear to be morphologically cryptic species.

The other most striking point of disagreement is the placement of A. conirostris—in the A. intermedius clade according to mtDNA and close to the A. tessellatus complex according to morphology. However, the mtDNA data support their placement of A. conirostris only weakly (branch support = 2, bootstrap < 50%), and this is the one node at which the MPTs of the combined analyses (Fig. 2) consistently differ from those of the mtDNA analysis.

Interpretation of Combined Data

It has been argued that if significant incongruence is found between two data sets, implying a difference in evolutionary history, the data should not be combined (Bull et al., 1993; de Queiroz et al., 1995). We agree that caution should be exercised in interpreting the results of heterogeneous combined data but argue that in some circumstances such results can be useful. In the case of Aramigus, we have a large number of mtDNA characters, reflecting maternal-lineage genealogy, and a much smaller number of morphological characters, reflecting some combination and interaction of maternal and paternal history (McDade, 1995). One might expect the results of the combined analysis in this case to primarily reflect maternal-lineage genealogy and to resemble the mtDNA results. Indeed, this is the case: Combined Analysis 3 and mtDNA Analysis 1 found three of the same trees, and the SW tree of Combined Analysis 2 conflicts with mtDNA Analysis 1 only in the relative positions of intermedius A and conirostris. One might even expect a combined analysis to provide a
better estimate of maternal-lineage genealogy than the mtDNA analysis, since the morphological characters contribute additional maternal-lineage “signal” along with paternal-lineage “noise.”

When relationships are reticulate, the problem of the extent to which morphological characters provide information on maternal or paternal ancestry is very complex (McDade, 1995; Rieseberg and Morefield, 1995). The extent to which morphological characters will provide information on maternal genealogy, paternal genealogy, both, or neither, depends on a number of factors. Consider three major factors relevant to this case:

1. The frequency of reticulation. If there is no reticulation, there is no distinction between maternal and paternal ancestry, and all morphological characters are equally informative of both. If reticulation is rare, there will be a distinction between maternal and paternal ancestry only in a few parts of the tree, and morphological characters will remain equally informative of both maternal and paternal genealogy across those regions of the tree where they are coincident. As reticulation becomes more rampant, it becomes rarer for a morphological character to be clearly informative of both maternal and paternal genealogy (since these are more rarely coincident), and it becomes more likely that the character will be informative of either maternal genealogy, or paternal genealogy, or neither.

2. Dominance. In the extreme case of a morphological character whose state is determined by a single locus with complete dominance, the character will be informative of either maternal or paternal genealogy, depending on which parent contributed the dominant allele. A morphological character that is determined by multiple loci, or by a locus with incomplete dominance, will tend be intermediate in hybrids—depending on how this character is coded for analysis, it might still be coded as having either the paternal state (and be hence informative of paternal genealogy) or the maternal state (hence be informative of maternal genealogy), or it might be coded as having a different state (hence informative of neither).

3. Sources of additional haploid genomes in polyploids. If only one of several haploid genomes in a polyploid lineage is originally from an egg, and the others are from sperm (as is likely in parthenogenetic weevils—see above), then morphological characters in hybrids will tend to be more informative of paternal than maternal genealogy. If all the additional haploid genomes were contributed by sperm from one sexual lineage, then the hybrid lineage might closely resemble this paternal lineage in many characters, and morphology might be strongly informative of paternal genealogy; however, if the additional haploid genomes were contributed by different sexual lineages, morphology might provide little recoverable information about either maternal or paternal genealogy.

Is it reasonable, then, to interpret the results of the combined analyses for Aramigus as informative of maternal-lineage genealogy? Because the sexual lineages, at least, are not of hybrid origin, and because some other features of the mtDNA and morphological results are in agreement (see Fig. 1), reticulation is apparently not utterly rampant across the entire phylogeny of Aramigus. Hence, morphological characters should indeed provide some “signal” of maternal-lineage genealogy—in those nonreticulating areas of the tree in which this is completely concordant with paternal-lineage genealogy, if nowhere else.

The question is whether the “noise” in the morphological characters with respect to maternal-lineage genealogy is unacceptable. If the noise were only the randomization or destruction of phylogenetic information (for instance, by the appearance of autapomorphies in hybrids [McDade, 1995]), the signal should outweigh it. What is more worrisome is that in some parts of the tree, there may be a strong competing signal of paternal genealogy. For instance, the detailed morphological similarity of the lineages bearing the highly divergent viridipallens A and viridipallens B haplotypes may plausibly be due to common paternal ancestry (Normark, 1994).
Clearly there are some non-hybrid lineages and some areas of agreement between mtDNA and morphological characters, and hence the morphological characters provide some information on maternal-lineage relationships. It is much less clear whether there is distinct and misleading paternal-lineage signal in the morphological characters, as opposed to mere confusing noise. Therefore, we interpret the tree shown in Figure 2 as our best estimate of maternal-lineage genealogy of Aramigus, at least within the A. tessellatus complex, where it is completely congruent with one of the 14 mtDNA MPTs.

**Phylogenetics of Parthenogenesis**

Evolutionary biologists have hypothesized a number of regularities in the evolution of parthenogenetic lineages—e.g., that in some taxa such as vertebrates, parthenogenetic lineages are always of hybrid origin, or that some cytogenetic classes of parthenogenetic lineages, such as polyploid amphiroids, are very unlikely to revert to sexuality (Bull and Charnov, 1985). The most significant of these proposed regularities, which underpins much current thinking about the adaptive significance of sex, is the hypothesis that parthenogenetic lineages are invariably doomed to rapid extinction (Hurst et al., 1992). This hypothesis remains controversial. One lineage of Aramigus, the “brown clade” of the A. tessellatus complex, has been suggested as a potentially ancient parthenogenetic lineage (Judson and Normark, 1996; Normark, 1996a), and hence that interpretation has been challenged (Little and Hebert, 1996). Here we consider the basis of this controversy.

**Standard model of the evolution of parthenogenesis.**—Obligately asexual lineages have long been viewed by evolutionists as ephemeral entities, arising frequently within many populations and rapidly undergoing extinction (Mayr, 1963; Maynard Smith, 1978; Bell, 1982; Futuyma, 1986). This “standard model” leads to a number of expectations: that extant parthenogenetic lineages will have accumulated few mutations since their origin, that ancestral sexual populations are either still extant or very recently extinct, and that apparently indistinguishable parthenogenetic lineages may turn out to have had separate origins. Even before relevant molecular-phylogenetic studies were available, the standard model was given fairly general credence by evolutionary biologists (Mayr, 1963; Maynard Smith, 1978; Futuyma, 1986). The model has been based on the perception that parthenogenetic lineages of animals usually have conspecific or congeneric sexual relatives, and it has also served important theoretical purposes. With its continual dynamics of origin and extinction, the model implies a close fit of any population’s actual genetic system to the optimal system for that population, and hence it has also inspired attempts to infer the adaptive significance of sexuality directly from observed ecological distributions of parthenogens vs. sexuals (Glesener and Tilman, 1978; Bell, 1982).

**Parthenogenetic lizards: the standard model corroborated.**—Molecular-phylogenetic studies of parthenogenetic lizards (and other unisexual vertebrates) have largely corroborated the standard model, finding generally low divergences between mtDNAs of extant parthenogenetic and sexual lineages, and often finding polyphyly of parthenogenetic taxa (Vyas et al., 1990; Avise et al., 1992; Moritz et al., 1992a, 1992b; Moritz, 1993). The identification of still-extant maternal and paternal sexual species (and often particular extant source populations) for almost every lineage of parthenogenetic lizards in recent years has been a remarkable success story in the annals of historical biology (Dawley and Bogart, 1989; Sites et al., 1990; Cole et al., 1993; Avise, 1994; Cole et al., 1995). Indeed, the success of this program has been so remarkable that some workers routinely presume that for every parthenogenetic lineage, ancestral sexual populations are extant. In some cases, the existence of a sexual parental taxon with particular characters has been hypothesized, and a taxon fitting the diagnosis has subsequently been found (Cole et al., 1993; Radtkey et al., 1995). In this research program, the process of finding and identifying extant sexual ancestral populations for parthenogenetic lineages is in some ways more similar in its spirit and methods to intrapopulational genealogical studies than to cladistic analysis.
The controversy over apparent counterexamples.—At the same time, other workers, often working on less intensively studied and exhaustively sampled taxa than the parthenogenetic vertebrates, have approached the phylogenetics of parthenogenetic lineages in a different way, more cladistic in methodology and agnostic in expectations. When mtDNA haplotypes have been found in parthenogens that are sharply divergent from those of any known sexual population, some authors have implicitly treated this as an unfortunate artifact of a failure to sample ancestral sexual populations, and have refrained from drawing attention to it (Turgeon and Hebert, 1994; Donnellan and Moritz, 1995; Van Raay and Crease, 1995). Others, however, have trumpeted the finding of high levels of divergence and have presented it as prima facie evidence of an ancient clonal lineage (Perez et al., 1994; Ó Foighil and Smith, 1995; Normark, 1996a; Ó Foighil and Smith, 1996). In the case of the nematode genus Meloidogyne, which includes a number of parthenogenetic crop pests, essentially similar molecular-phylogenetic patterns were initially interpreted by one set of authors as suggesting multiple recent origins of parthenogenesis (Hugall et al., 1994), and by another as suggesting one ancient origin (Castagnone Sereno et al., 1993). However, the distinction between these two research traditions may be eroding: In the most recent discussions (including the present one) some of these same authors address more explicitly the difficulty of distinguishing ancient from recent origins of parthenogenesis, and refrain from drawing firm conclusions (Chaplin and Hebert, 1997; Hugall et al., 1997; Little and Hebert, 1997).

From the two historically different perspectives, the evolution of parthenogenesis in Aramigus is susceptible to profoundly different interpretations. If the standard model of the evolution of parthenogenesis is assumed a priori to be correct, the data imply that a large number of extant sexual lineages have gone unsampled. In Figure 5, the maternal sexual lineages expected under the standard model have been added to the cladogram; a precedent for inclusion of theoretically expected lineages in cladograms is found in the reconciled trees used in biogeography and other contexts (Nelson and Platnick, 1981; Page and Charleston, 1997). If, in contrast, the number of process assumptions is minimized, as in a fairly standard cladistic analysis (Fig. 4), the evolution of parthenogenesis looks radically different from that postulated by the standard model. There are a few lines of circumstantial evidence that support the standard model (Fig. 5) as more plausible than the simple cladistic result (Fig. 4) for Aramigus. The evidence supporting hybrid (and therefore sexual) origins of parthenogenetic lineages in Aramigus, discussed above under incongruence, falls in this category. So does evidence that sexual lineages are likely to have been systematically undersampled: Known sexual populations of Aramigus have smaller geographical ranges than do the parthenogens to which they are most closely related (Lanteri and Normark, 1995). This is a common pattern in weevils and many other taxa (Vandel, 1928; Lynch, 1984) and tends to bias sampling against sexual lineages. One of the sexual populations in Aramigus, sexual A. uruguayensis, was discovered only in the course of the molecular-phylogenetic study and is still known from only one site (Normark and Lanteri, 1996). And yet, since we would ultimately like to test the standard model of the evolution of parthenogenesis using phylogenetic data, we cannot assume it to be correct a priori, as in Figure 5. One alternative to the extreme dichotomy between Figures 4 and 5 is to consider other hypotheses regarding the evolution of parthenogenesis, not those we are seeking to test, that could be used to inform, or constrain, its optimization on the tree.

Irreversibility.—Evolutionary transitions from sexuality to some forms of parthenogenesis, and from diploidy to some forms of polyploidy, have been hypothesized to be irreversible (Bull and Charnov, 1985). Aramigus exemplifies the sort of transition that is expected to be especially resistant to reversal: from a male-heterogametic, dioecious sexual system to polyploid (usually triploid) apomorphic system. Restoration of the ancestral sexual system in such cases would require a successful asymmetric meiosis to separate one haploid genome from two or more others, along with reacquisition of a male-determining sys-
Figure 5. A tree based on the tree in Figure 2, to which hypothetical maternally ancestral sexual lineages have been added to illustrate the expectations of the standard model of the evolution of parthenogenesis (white = sexual, black = parthenogenetic) is the same whether or not the irreversibility assumption is invoked.
tem (either de novo or by outcrossing to “capture” a Y chromosome from a related sexual species). Triploid animals generally seem to have zero success at producing viable haploid gametes (Lynch, 1984; Dawley et al., 1985; Kurita et al., 1995); however, the ability of some triploid plants to produce haploid gametes shows that it is possible (Felber and Bever, 1997). Mating with related sexual lineages could in principle raise ploidy level to an even number, making meiosis slightly less problematic, but the extreme rarity of sexual polyploid animals suggests that this does not happen, and the egg would still not be haploid. Perhaps the most plausible scenario is of a triploid apomict successfully undergoing meiosis to produce a haploid egg and also mating with a related sexual lineage. The origin of an apomictic lineage from a sexual one, in contrast, requires only a failure of meiosis to occur (as in a hybrid), followed by a triggering of development in the absence of synergamy (easily accomplished in vitro for some taxa by any of various treatments (Suomalainen et al., 1987)). Hence, cytogenetic considerations suggest that in Aramigus, transitions from sexuality to parthenogenesis are much simpler, and probably occur much more frequently, than reversions from parthenogenesis to sexuality.

We can use this cytogenetic information in our character optimization. Though reversions from parthenogenesis to sexuality may be possible in principle, we can code the character as irreversible, as a first approximation, reflecting the relatively great expected rarity of reversion (Fig. 3). By optimizing more internal nodes as sexual, the irreversibility assumption provides a crude correction for likely undersampling of sexual lineages. Because hypotheses of ancient parthenogenesis are controversial—both for theoretical reasons and because a number of such hypotheses have subsequently been falsified by the discovery of sexual populations (Judson and Normark, 1996)—this conservatism is desirable. And yet, assuming irreversibility is much less onerous than assuming the validity of the standard model a priori. Because the irreversibility assumption relies only on cytogenetic considerations, its use does not preclude falsifying the standard model.

**Conclusion and Prospects**

Our results lend further indirect support to the hypothesis that hybridization is important in the origins of polyploid parthenogenetic weevil lineages. This hypothesis requires more direct testing. In many cases, particularly in parthenogenetic vertebrates, hypotheses of hybrid origin have been convincingly corroborated by allozyme studies. The hybridity of a lineage is corroborated by showing that it shares identical electromorphs at several or many loci with each of two distinct sexual populations (Dawley and Bogart, 1989). Hence this method of corroborating using allozymes depends upon the existence and availability of at least two sexual populations to serve as candidate parental taxa. It also requires identity between lineages of electromorphs at variable loci, and hence relies upon the recency of origin of the hybrid lineage. In parthenogenetic vertebrate lineages, this recency of origin has in many cases been corroborated by the discovery that the parthenogenetic lineage shares an identical or nearly identical (< 2% divergent) mtDNA haplotype with one of the parental sexual populations (Avise et al., 1992; Moritz et al., 1992a; Moritz, 1993).

This method of corroborating hybrid origins by using allozymes seems likely to be problematic in Aramigus. First, there is a paucity of candidate parental sexual taxa. Within the A. tessellatus complex, only one sexual lineage is known. Second, there are no candidate maternal sexual taxa. The parthenogenetic lineages of A. tessellatus are highly (4.0% to 7.3%) divergent from the sexual one in mtDNA (cytochrome oxidase I) sequence, and hence are not consistently preserved. Hence, to test the hypothesis that the incongruence between morphological and mtDNA data in Aramigus results from hybrid origins of parthenogenetic lineages, the best prospect is sequence data from nuclear genes. Nuclear sequences have now been used successfully to corroborate hybrid origins of a number of lineages, mostly of plants (Rieseberg and Morefield, 1995; Soltis and Kuzoff, 1995; Schilling and Panero, 1996), but also of at
At least one vertebrate (Schartl et al., 1995). Nuclear gene sequences also have great promise for resolving questions about the antiquity and evolution of parthenogenetic lineages (Birky, 1996; Judson and Normark, 1996).

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APPENDIX 1

List of morphological characters and character states, modified from Lanteri and Díaz (1994).
APPENDIX 2. Morphological characters for *Anmmigus* (modified from Lanteri and Díaz, 1994).

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