

## Correlated Evolution in Fig Pollination

GEORGE D. WEIBLEN

Department of Plant Biology, University of Minnesota, 1445 Gortner Avenue, Saint Paul, Minnesota 55108, USA;  
E-mail: gweiblen@umn.edu

**Abstract.**—This is the first comparative study of correlated evolution between figs (*Ficus* species, Moraceae) and their pollinators (Hymenoptera: Agaoninae) based on molecular phylogenies of both lineages. Fig relationships based on the internal transcribed spacer region (ITS) of nuclear ribosomal DNA and pollinator relationships inferred from mitochondrial cytochrome oxidase I (COI) sequences enabled the study of correlated evolution based on molecular phylogenies for the largest set of interacting species ever compared. Comparative methods have been applied to tests of adaptation, but the application of these methods in tests of coadaptation, defined as reciprocal evolutionary change in interacting lineages, has received less attention. I have extended tests of correlated evolution between two traits along a phylogeny to the case of interacting lineages, where two traits may or may not share a common phylogenetic history. Independent contrasts and phylogenetic autocorrelation rejected the null hypothesis that trait correlations within lineages are stronger than trait correlations between interacting lineages. Fig style lengths and pollinator ovipositor lengths, for example, were more highly correlated than were pollinator body size and ovipositor length. Mutualistic interactions between figs and their pollinators illustrate the novel ways in which phylogenies and comparative methods can detect patterns of correlated evolution. The most outstanding evidence of correlated evolution between these obligate mutualists is that interacting trait correlations are stronger than within-lineage allometric relationships. [Agaonidae; coadaptation; coevolution; comparative methods; *Ficus*; mutualism.]

Coevolution of the obligate mutualism between figs (*Ficus* species, Moraceae) and their pollinators (Hymenoptera: Agaoninae) has not been examined in a twin phylogenetic framework until now. The emergence of phylogeny estimates for these plants and their obligate pollinators (Yokoyama, 1995; Herre et al., 1996; Machado et al., 1996, 2001; Weiblen, 2000, 2001) makes it possible to test hypotheses of coevolution in a system of long-standing interest (Janzen, 1979; Futuyma and Slatkin, 1983). Taxonomists were the first to suggest that figs and pollinators may have cospeciated (Wiebes, 1979; Corner, 1985), a pattern of association now supported by molecular phylogenetic evidence drawn from each partner (Weiblen and Bush, 2002). Much of the recent interest in the evolution of interactions has focused on cospeciation, but cospeciation is neither necessary nor sufficient evidence of reciprocal adaptation in two interacting lineages, a key feature of the coevolutionary process (Page, 2003). In parasitic interactions, for example, the correlated evolution of virulence and resistance is predicted whether or not phylogenies of the parasites and hosts are identical. Here, I have evaluated hypotheses of correlated evolution between fig and pollinator traits.

The first predictions of fig/pollinator coadaptation concerned closely interacting morphological traits (Ramirez, 1978, 1980; Wiebes, 1979; Murray, 1985; Berg, 1990). For example, pollinators lay eggs in fig flowers by piercing styles with their ovipositors, and the style length distribution of a fig species might select for an optimal ovipositor length in its pollinator (Ganeshaiah et al., 1995). Conversely, the impact of pollinators galling fig ovules could select for style lengths that strike a balance between the production of seeds and the rearing of pollen vectors (Bronstein, 1988). As predicted, there is a strong association between style lengths and ovipositor lengths among interacting species pairs (Murray, 1985; Nefdt and Compton, 1996), but phylogenetic comparisons have not been possible until now.

In monoecious figs, female agaonine wasps lay eggs in a fraction of the flowers they pollinate, such that viable seeds and pollinator offspring reach maturity in the same fig (Fig. 1). Theoretically, ovipositors long enough to access all flowers would be selected (Murray, 1985) but an efficiency cost to egg laying in long styles would impose a limit on optimal ovipositor length (Ganeshaiah et al., 1995). Studies have shown that ovipositors can penetrate even the longest styles in monoecious figs but that most egg laying occurs in flowers with shorter styles (Nefdt and Compton, 1996; Jousselein et al., 2001). Given time constraints on oviposition due to short lifespans or intraspecific competition, a preference for short-styled flowers is predicted from optimal foraging theory (D. Yu, pers. comm.).

Functionally dioecious fig species, however, have divided the production of seeds and the rearing of pollinators into two inflorescence types on separate plants, called gall figs and seed figs (Fig. 1). Pollinator ovipositors are long enough to pierce the short styles of gall figs but are too short for the longer styles of seed figs (Weiblen et al., 2001). Paradoxically, pollinators show little or no preference for gall figs. Each larva destroys a single seed in gall figs, but seed figs are unharmed and represent a dead end in terms of pollinator fitness (Kjellberg et al., 1987). Why have these pollinators not evolved longer ovipositors enabling them to colonize seed figs or not learned to avoid seed figs altogether? The persistence of mutualism in 350 species of functionally dioecious *Ficus* has intrigued fig biologists (Grafen and Godfray, 1991; Kerdelhué and Rasplus, 1996; Anstett et al., 1997; Weiblen et al., 2001; Harrison and Yakamura, 2003).

In this study, I examined correlations among characters, such as fig breeding systems and pollinator ovipositor lengths, hypothesized to be products of coadaptation (Ramirez, 1980; Berg, 1990). Testing such hypotheses in a phylogenetic framework is preferable to treating species as independent data points because of

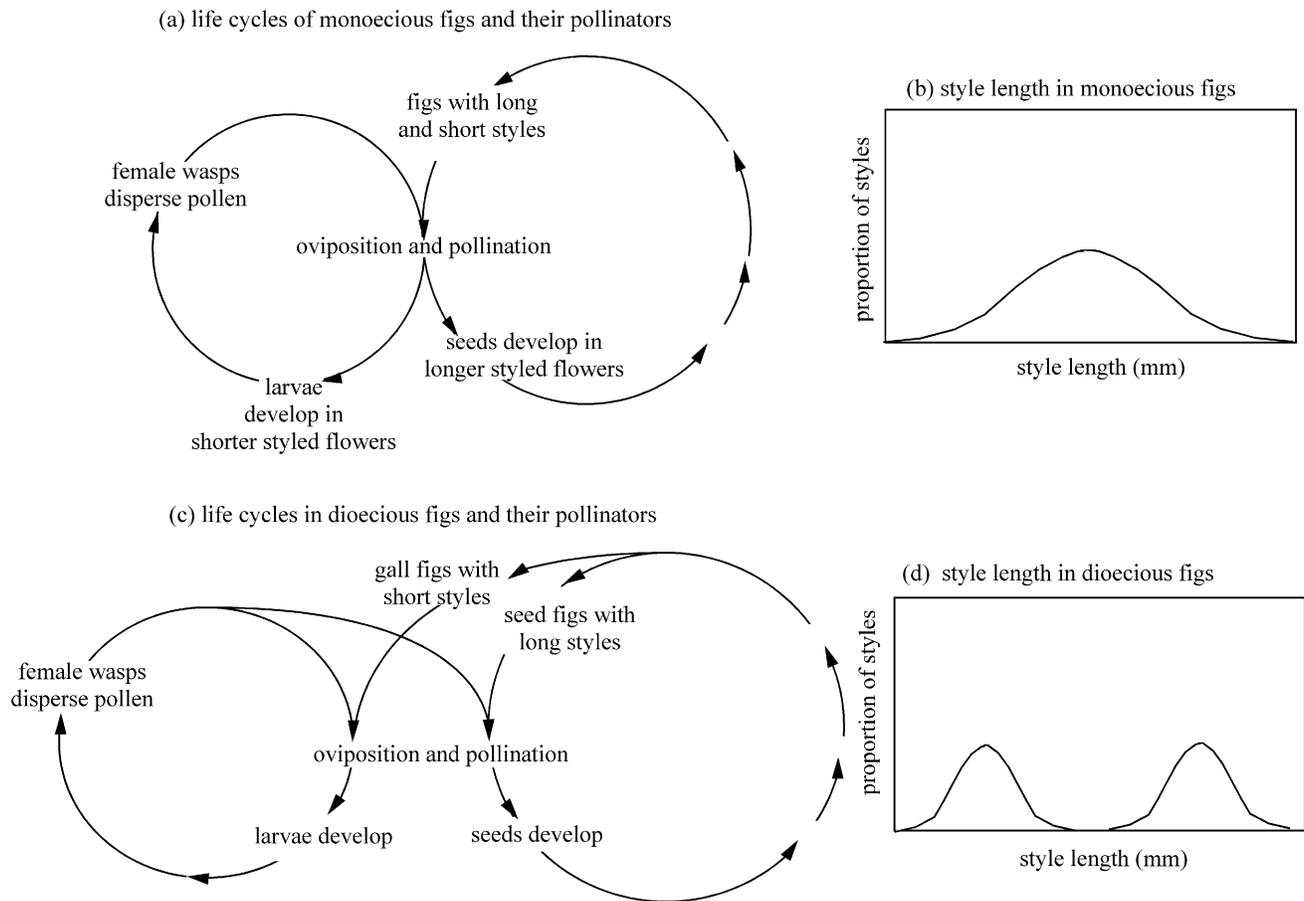


FIGURE 1. Life cycles of monoecious and functionally dioecious *Ficus* and their pollinators. (a) In monoecious species, seeds and pollinators develop in the same fig. (b) The distribution of style lengths in monoecious figs is unimodal, but pollinators tend to develop in shorter styled flowers and seeds tend to develop in longer styled flowers. (c) In functionally dioecious species, the production of pollinators and seeds is segregated in two types of figs on separate plants. (d) Style length dimorphism divides the maturation of pollinators and seeds into gall figs (first mode) and seed figs (second mode), respectively.

the potential for inflated or spurious correlations (Nefdt and Compton, 1996; van Noort and Compton, 1996). Comparative methods can be used to evaluate evolutionary scenarios, including hypotheses of adaptation (Coddington, 1988; Baum and Larson, 1991; Frumhoff and Reeve, 1994; Leroi et al., 1994; Losos and Miles, 1994; Martins, 2000), sequences of character change (Donoghue, 1989), and correlations among characters (Maddison, 1990). A common approach to evaluating an adaptive hypothesis involves testing the significance of the association between a trait that varies among a group of related species (e.g., body size) and variation in the environment (e.g., temperature). Bearing in mind that correlation does not establish causation, comparative analyses can at least support an adaptive hypothesis and point to definitive experiments.

Statistical techniques incorporating phylogenetic information, such as the method of independent contrasts (Felsenstein, 1985), were developed to evaluate the strength of correlation between two traits in a lineage. Such approaches have rarely been used to examine hypotheses of reciprocal adaptation between two

lineages (Morand et al., 2000; Clayton et al., 2003). Evidence for the correlated evolution of interacting traits has supported hypotheses of antagonistic coevolution (Arnqvist and Rowe, 2002) but has not been extended to cases in which traits have different histories. Here, I outline how comparative methods may be applied to the study of interspecific coadaptation with examples from fig pollination. In particular, independent contrast analysis (Felsenstein, 1985) and phylogenetic autoregression (Martins and Garland, 1991; Garland et al., 1992) were used to test hypotheses of correlated evolution in continuous traits.

## MATERIALS AND METHODS

### *Taxon Sampling*

Sampling was based on pairs of fig and pollinator species from previous phylogenetic analyses (Weiblen, 2000, 2001), being limited to representatives of major taxonomic groups (most sections of *Ficus* and genera of Agaoninae; Table 1). Dioecious figs and their pollinators were sampled most intensively, especially hosts

TABLE 1. Associations (numbered) between *Ficus* species and their pollinating Agaoninae included in comparative analyses of correlated evolution. Three substitutions are shown in parentheses. Pollinator subgenera are (C)*eratosolen*, (P)*arapristina*, (R)*othropus*, (S)*trepitus*, and (V)*alisia*.

Association	Pollinator	<i>Ficus</i> sections	<i>Ficus</i> species
1	<i>Blastophaga</i> (V.) <i>intermedia</i>	<i>Ficus</i>	<i>padana</i>
2	<i>Blastophaga</i> (V.) <i>malayana</i>	<i>Ficus</i>	<i>grossularioides</i>
3	<i>Ceratosolen</i> (C.) <i>appendiculatus</i>	<i>Neomorphe</i>	<i>variegata</i>
4	<i>Ceratosolen</i> (C.) <i>bisulcatus</i>	<i>Sycocarpus</i>	<i>septica</i>
5	<i>Ceratosolen</i> (C.) <i>blommersi</i>	<i>Sycomorus</i>	<i>botryoides</i>
6	<i>Ceratosolen</i> (C.) <i>capensis</i>	<i>Sycomorus</i>	<i>sur</i>
7	<i>Ceratosolen</i> (C.) <i>waliensis</i>	<i>Neomorphe</i>	<i>robusta</i>
8	<i>Ceratosolen</i> (C.) <i>emarginatus</i>	<i>Neomorphe</i>	<i>auriculata</i>
9	<i>Ceratosolen</i> (C.) <i>fusciceps</i>	<i>Neomorphe</i>	<i>racemosa</i>
10	<i>Ceratosolen</i> (C.) <i>grandii</i>	<i>Adenosperma</i>	<i>semivestita</i>
11	( <i>Ceratosolen</i> (C.) <i>medlerianus</i> )	<i>Adenosperma</i>	<i>adenosperma</i>
12	<i>Ceratosolen</i> (C.) <i>nanus</i>	<i>Sycocarpus</i>	<i>pungens</i>
13	<i>Ceratosolen</i> (C.) <i>nexilis</i>	<i>Neomorphe</i>	<i>nodosa</i>
14	<i>Ceratosolen</i> (C.) <i>riparianus</i>	<i>Adenosperma</i>	<i>ochrochlora</i>
15	<i>Ceratosolen</i> (R.) <i>corneri</i>	<i>Sycocarpus</i>	<i>botryocarpa</i>
16	<i>Ceratosolen</i> (R.) <i>dentifer</i>	<i>Sycocarpus</i>	<i>hispidioides</i>
17	<i>Ceratosolen</i> (R.) <i>hooglandi</i>	<i>Sycocarpus</i>	<i>bernaysii</i>
18	<i>Ceratosolen</i> (S.) <i>abnormis</i>	<i>Sycocarpus</i>	<i>dammarsopsis</i>
19	<i>Ceratosolen</i> (S.) <i>armipes</i>	<i>Sycocarpus</i>	<i>itoana</i>
20	<i>Ceratosolen</i> (S.) <i>kaironkensis</i>	<i>Sycocarpus</i>	<i>microdictya</i>
21	<i>Ceratosolen</i> (S.) <i>vissali</i>	<i>Sycocarpus</i>	<i>theophrastoides</i>
22	<i>Dolichoris inornata</i>	<i>Oreosycea</i>	<i>edelfeltii</i>
23	<i>Dolichoris subtrinervia</i>	<i>Oreosycea</i>	<i>subtrinervia</i>
24	( <i>Dolichoris vasculosae</i> )	<i>Oreosycea</i>	<i>albipila</i>
25	<i>Eupristina</i> (P.) <i>verticillata</i>	<i>Conosycea</i>	<i>microcarpa</i>
26	<i>Kradibia copiosae</i>	<i>Sycidium</i>	<i>copiosa</i>
27	<i>Kradibia jacobsi</i>	<i>Sycidium</i>	<i>conocephalifolia</i>
28	<i>Kradibia ohuensis</i>	<i>Sycidium</i>	<i>trachypison</i>
29	<i>Kradibia salembensis</i>	<i>Sycidium</i>	<i>phaeosyce</i>
30	<i>Kradibia wassae</i>	<i>Sycidium</i>	<i>wassa</i>
31	<i>Liporrhopalum</i> cf. <i>gibbosae</i>	<i>Sycidium</i>	<i>tinctoria</i>
32	<i>Liporrhopalum virgatae</i>	<i>Sycidium</i>	<i>virgata</i>
33	<i>Platyscapa corneri</i>	<i>Urostigma</i>	<i>superba</i>
34	<i>Platyscapa fischeri</i>	<i>Urostigma</i>	<i>prasinicarpa</i>
35	<i>Pleistodontes plebejus</i>	<i>Malvanthera</i>	<i>hesperidiiformis</i>
36	<i>Pleistodontes rieki</i>	<i>Malvanthera</i>	<i>xylosycea</i>
37	<i>Pleistodontes rigisamos</i>	<i>Malvanthera</i>	<i>destruens</i>
38	( <i>Tetrapus costaricanus</i> )	<i>Pharmacosycea</i>	<i>insipida</i>
39	<i>Waterstoniella brevigena</i>	<i>Conosycea</i>	<i>pellucidopunctata</i>
40	<i>Wiebesia punctatae</i>	<i>Kalosyce</i>	<i>punctata</i>
41	<i>Wiebesia brusi</i>	<i>Rhizocladus</i>	<i>baeuerlenii</i>
42	<i>Wiebesia frustrata</i>	<i>Rhizocladus</i>	<i>odoardi</i>

and members of the genus *Ceratosolen*. *Ficus* sequences from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) and fig wasp sequences from the cytochrome oxidase I (COI) gene of mitochondrial DNA (mtDNA) were available for 39 pairs of interacting fig and pollinator species. The inclusion of three additional pairs served to better approximate the diversity of monoecious fig and pollinator clades for subsequent analyses of fig breeding system evolution. In particular, *Tetrapus costaricanus* was paired with monoecious *F. insipida* in sect. *Pharmacosycea* (Herre et al., 1996). The pollinator of *F. albipila* (sect. *Oreosycea* ser. *Vasculosae*) was not available, and *Dolichoris vasculosae*, the pollinator of closely related *F. vasculosa*, was substituted. Although, mtDNA was not available for *Ceratosolen adenospermae*, morphology is indicative of a close relationship with *C. medlerianus*, and this species was paired with *F. adenosperma* to represent the pollinators of sect. *Adenosperma* (Wiebes, 1981). Phylogenetic

hypotheses for a total of 42 interacting species pairs were published trees from parsimony analyses of DNA sequences (Weiblen, 2000, 2001). These analyses and similar results obtained by maximum likelihood (ML) were detailed previously (Weiblen, 2000, 2001). Pollinator phylogeny was inferred from 1,029 parsimony informative mtDNA characters from 1,774 aligned nucleotide positions. Fig phylogeny was based on 174 nrDNA characters from 776 positions in total. Bootstrap support for relationships was obtained from heuristic searches with 10 random addition sequence replicates, a maximum of 10,000 trees per replicate, and 100 bootstrap replicates.

#### Trait Measurements

Species means and SEs were obtained from specimens listed by Weiblen (1999) or from the literature for *F. sur* and *Ceratosolen capensis* (Bajinath and Ramcharun, 1983; Nefdt and Compton, 1996), *F. variegata* (Weiblen et al.,

1995), *F. racemosa* and *C. fusiceps* (Kathuria et al., 1995), and *F. botryoides* (Berg, 1986). Each trait except fig wall thickness was measured to the nearest 0.01 mm using an Olympus SZH10 stereo microscope and a Polaroid digital microscope camera.

The influence of fig developmental phase is important to consider in functional comparisons with ovipositor lengths. Pollinator ovipositors penetrate styles during a brief period of receptivity, and the variance in style length increases to accommodate the enlarging flowers later in development (Verkerke, 1988). Style lengths were measured in ripe figs from which pollinators were reared, owing to unavailability of sufficient material in receptive phase. These measurements overestimated style lengths at the receptive phase, but style length can also underestimate the distance from the stigmatic surface to the site of egg deposition, which lies between the integument and the nucellus of the fig ovule (Cunningham, 1888; Ganeshiah et al., 1995). On balance, these considerations do not suggest a systematic bias in comparisons among species. Pollinator ovipositors were measured from the first and second valvulae because the sheaths (third valvula) underestimate the length of the functional ovipositor (Nefdt and Compton, 1996).

Fig wall thickness, gall size, and wasp body size were measured to enable tests of additional hypotheses of correlated evolution, such as gall size in relation to wasp body size (Nefdt and Compton, 1996) and fig wall thickness in relation to ovipositor length (Compton and Nefdt, 1988). Dried fig measurements were multiplied by a factor of 1.67 to approximate the fresh condition (Laman and Weiblen, 1998). Thorax length was used as a relative indicator of wasp body size because thorax length is strongly correlated with overall body length and is easier to measure (S. Compton, pers. comm., 1997). The width of mature gall ovaries was compared with fig wasp body size, owing to the different sizes of gall ovaries and achenes (Verkerke, 1988). Measurements from 24 nonpollinating *Sycoscapter* species (*Sycoryctinae*; mostly undescribed) associated with the 42 fig species were also compared with fig traits. Models for *Sycoscapter* trait evolution were fitted to pollinator phylogenies based on evidence that nonpollinators have cospeciated with pollinators (Machado et al., 1996; Lopez-Vaamonde et al., 2001).

#### *Tests of Correlated Evolution in Discrete Characters*

Correlated change in discrete morphological traits was examined using parsimony and ML methods of ancestral state reconstruction (Maddison, 1990; Pagel, 1994). Ancestral fig breeding systems and relative ovipositor lengths were reconstructed on fig and pollinator molecular phylogenies in MacClade (Maddison and Maddison, 1992). Ovipositor length relative to the thorax was split into two discrete states, owing to the bimodal distribution of this trait (Fig. 2). Long ovipositors were considered to be equal to or more than the length of the thorax and short ovipositors were considered to be shorter than the thorax.

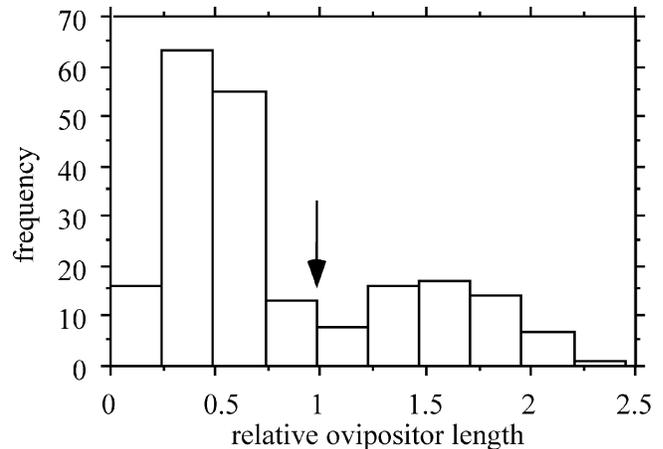


FIGURE 2. Bimodal distribution of ovipositor length relative to thorax length for 38 pollinator species and 205 individuals (141 dioecious fig pollinators and 69 monoecious fig pollinators). Relative ovipositor length was divided (arrow) into two discrete states, long and short. Long ovipositors were defined as those equal to or more than the length of the thorax (ratio of 1.01–2.45), and short ovipositors were defined as being shorter than the thorax (ratio of 0.11–0.95).

Maddison's (1990) concentrated changes test addressed the issue of whether changes in a dependent trait were significantly associated with clades bearing a particular state of an independent character. The test was applied to fig and pollinator phylogenies in a reciprocal manner by reversing the dependence of the variables in each case. With respect to fig phylogeny, for example, breeding system was a dependent variable and relative ovipositor length was treated as an independent feature of the environment. The test involved calculating the probability of obtaining by chance the observed number of gains and losses of functional dioecy in fig clades pollinated by wasps with short ovipositors. The reciprocal test assumed breeding system to be independent of ovipositor length and pollinator phylogeny. The extent of cospeciation does not influence the theoretical predictions. For instance, if a pollinator switched from a monoecious fig to a dioecious fig, a corresponding change in ovipositor length is predicted in response to the new environment.

ML tests for correlated evolution between breeding system and relative ovipositor length were also computed with the program Discrete (Pagel, 1994, 1997). ML branch lengths for fig and pollinator topologies were estimated from nrDNA and mtDNA under general time reversible (GTR) models of molecular evolution with the addition of parameters for heterogeneity in the rate of substitution across sites (GTR+ $\Gamma$ ), the best fitting models available for these data (Weiblen and Bush, 2002). An omnibus test of correlated evolution was conducted for each tree according to the reciprocal procedure outlined above. The test compared the log-likelihood ratio (LR) of an unrestricted eight-parameter model to that of a four-parameter model in which rates of change in the two discrete characters were constrained to be equal (Pagel, 1994, 1997). Because these models were not nested,

a distribution of the LR was generated by Monte Carlo simulation with 100 replicates under the null hypothesis of character independence. Whether changes in ovipositor length preceded changes in breeding system or vice versa was tested by comparing the  $\chi^2$ -distributed LR of the eight-parameter model to that of a seven-parameter restricted model, following Pagel (1994).

#### *Tests of Correlated Evolution in Continuous Characters*

Methods for reconstructing continuous character evolution are preferable to partitioning traits such as ovipositor length into discrete states (Fig. 2). Ancestral ovipositor lengths reconstructed on the fig phylogeny using the phylogenetic generalized least squares (PGLS) method of Martins and Hansen (1997) enabled the comparison of inferred ancestral ovipositors associated with monoecious and dioecious sister groups of figs. The PGLS method as implemented in the computer program COMPARE (Martins, 2001) has the advantages of considering intraspecific variation in the model of character evolution and of assigning error terms to ancestral values.

Correlations between continuous traits were also examined with the method of independent contrasts (Felsenstein, 1985). ML branch lengths were calculated for each data set assuming GTR +  $\Gamma$  and parameter estimates for fig and pollinator topologies separately. Contrasts were divided by the SD of expected change along each branch to satisfy parametric statistical assumptions (Martins and Garland, 1991), and regressions were constrained to pass through the origin, because of the arbitrary sign of each contrast (Garland et al., 1992). Fig and fig wasp traits were tested against their respective topologies by reversing the dependence of the variables.

Phylogenetic autocorrelation methods also tested for evolutionary correlations between fig and wasp traits. COMPARE was used to fit general linear models of interspecific variation in fig and wasp traits on their respective phylogenies. Residuals represent species variation that is not explained by a model of phylogenetic inertia, and a significant relationship between residual values for two traits is evidence of correlated evolution. An advantage of autocorrelation over independent contrasts is that residuals based on different topologies for fig and wasp traits can be compared simultaneously. Fig and pollinator topologies based on combined analyses of morphological and molecular data (Weiblen, 2000, 2001) were used to examine the sensitivity of results to phylogenetic uncertainty (Donoghue and Ackerly, 1996).

## RESULTS

### *Correlated Evolution in Discrete Characters*

The evolution of fig breeding system was closely associated with ancestral changes in the relative length of pollinator ovipositors. Four shifts in breeding system and five shifts in ovipositor length are indicated in Figure 3. Two independent origins of dioecy in figs were associated with reduced ovipositor length, and each reversal

from dioecy to monoecy was associated with a shift to longer ovipositors. Correlated evolution of these traits was supported by ML omnibus tests (Pagel, 1994) with respect to fig and pollinator molecular phylogenies. In the case of fig phylogeny, changes in breeding system were significantly correlated with changes in ovipositor lengths of the associated pollinators (LR = 11.3, df = 4,  $P = 0.01$ ). In the pollinator phylogeny, shifts in ovipositor length were correlated with shifts in fig breeding system (LR = 9.3, df = 4,  $P = 0.01$ ).

Concentrated changes tests based on exact searches under parsimony were similar for fig and pollinator topologies. The probability of obtaining by chance two gains and two losses of dioecy in fig clades pollinated by wasps with short ovipositors was not significant ( $P = 0.21$ ). Similarly, gains and losses of short ovipositors were not significantly concentrated in clades pollinating dioecious figs ( $P = 0.20$ ). It is not surprising that tests for concentrated change failed to detect significance given the low incidence of change and the fact that most branches were reconstructed as dioecious or as having short ovipositors. Contingent states tests (Sillen-Tulberg, 1993) yielded similar results. Changes in fig breeding system and ovipositor length coincided so closely that log-likelihood tests failed to detect the temporal order or sequence of evolutionary change. For example, the rate of change to short ovipositors in pollinators of monoecious lineages was not significantly different from the rate of change to dioecy in lineages pollinated by short-ovipositor wasps (LR = 2.20, df = 1). The only case in which changes were not concurrent involved the relatively short ovipositors of *Pleistodontes* pollinating monoecious *Malvanthera* figs.

### *Correlated Evolution in Continuous Characters*

Correlated evolution was also evident for continuous characters. Each sister group comparison of ovipositor length of pollinators associated with monoecious and dioecious fig clades was consistent with the predicted direction of change; relative reduction (and elongation) of the ovipositor was associated with the gain (and loss) of dioecy (Fig. 4). For example, there was a reduction in ovipositor length in the ancestral pollinators of dioecious subg. *Ficus* relative to the monoecious sister group, sect. *Urostigma*. Similarly, inferred ancestral ovipositor lengths of pollinators of dioecious subg. *Sycomorus* were reduced compared with those of pollinators of the monoecious sect. *Oreosycea*. Each reversal from dioecy to monoecy accompanied the elongation of the ovipositor in the associated pollinator lineage. A fifth contrast (Fig. 4e) not included in Figure 3 but based on morphological evidence (Wiebes, 1963) also showed a significant increase in ovipositor length with the loss of dioecy.

The pattern is illustrated by correlated trait changes in *Ceratosolen armipes*, *C. kaironkensis*, and their respective hosts (Fig. 5). Shifts from style length dimorphism, as in functionally dioecious *F. itoana*, to a unimodal distribution, as in *F. microdictya*, were accompanied by a significant increase in ovipositor length. Ovipositors of

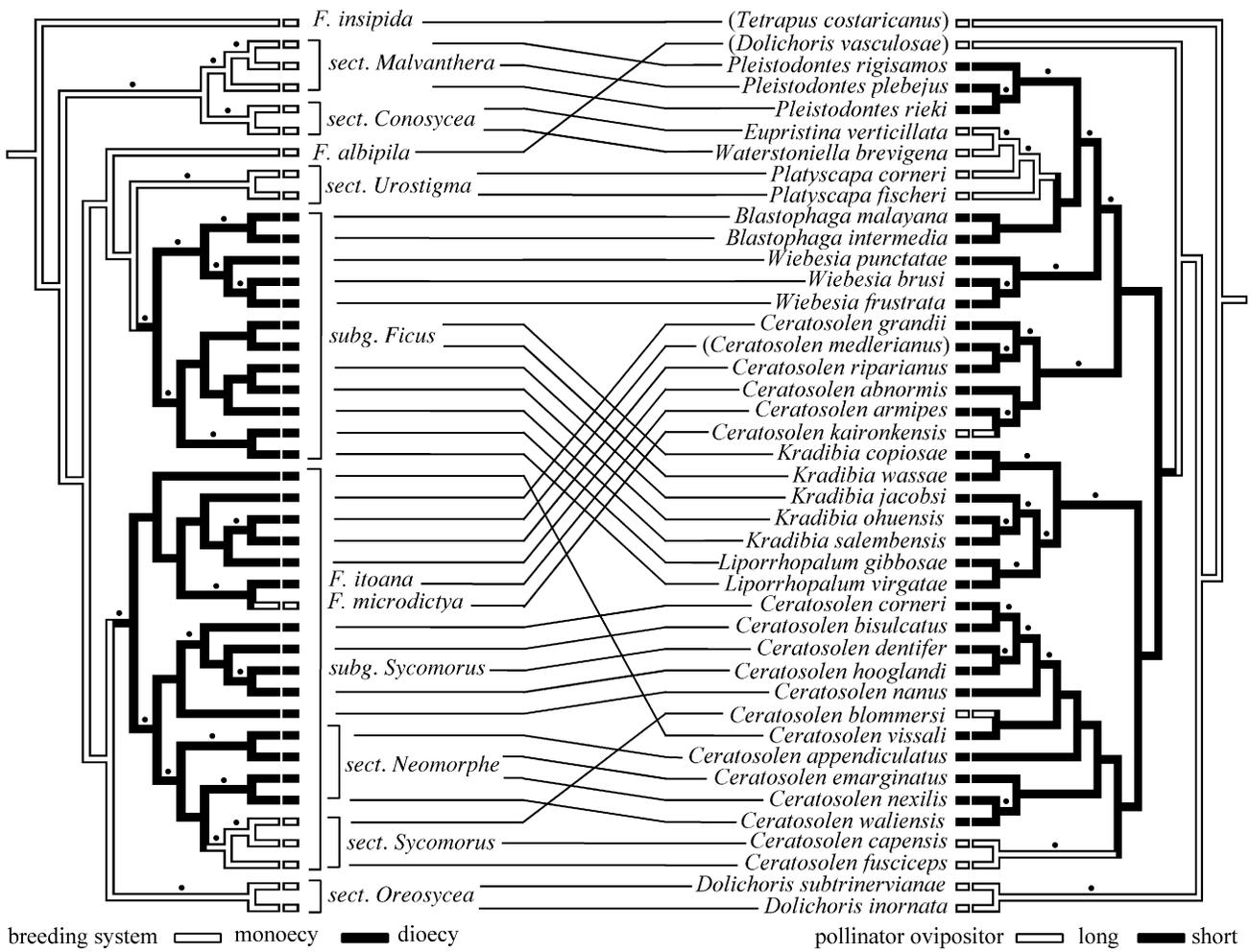


FIGURE 3. Fig and pollinator phylogenies indicating current associations and ancestral reconstructions of fig breeding systems and pollinator ovipositor lengths. Most-parsimonious trees for 42 fig species and their pollinators are based on separate analyses of nrDNA and mtDNA sequences, respectively (Weiblen, 2000, 2001). Bootstrap values >70% are indicated by dots above the branches. Brackets mark some *Ficus* sections and subgenera, and three substituted pollinators are indicated in parentheses.

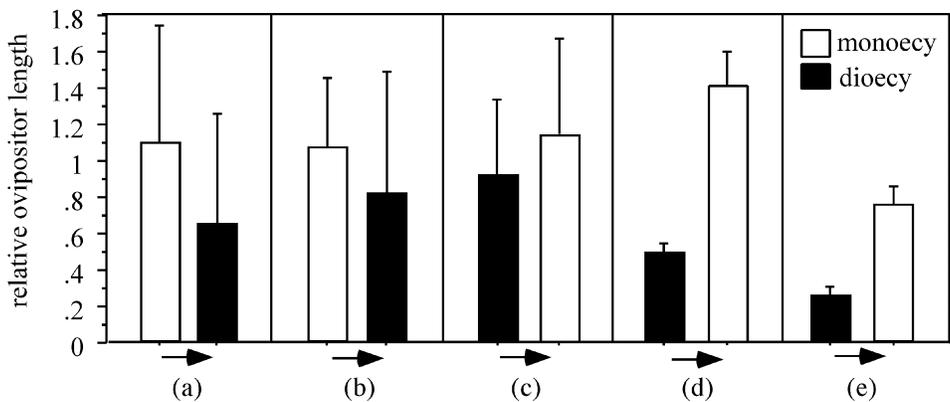


FIGURE 4. Sister-group comparisons of relative mean ovipositor length ( $\pm$ SE) in pollinators of monoecious and dioecious figs. Arrows indicate the direction of inferred changes in breeding system, whether dioecy was gained or lost. (a) Pollinators of monoecious sect. *Urostigma* and dioecious subg. *Ficus*. (b) Pollinators of monoecious sect. *Oreosycea* and dioecious subg. *Sycomorus*. (c) Pollinators of dioecious sect. *Neomorphe* and monoecious sect. *Sycomorus*. (d) Pollinators of dioecious *F. itoana* and monoecious *F. microdictya*. (e) Pollinators of dioecious *F. pungens* and monoecious *F. pritchardii* were contrasted based on morphological evidence that they represent a fifth contrast (Wiebes, 1963). Ancestral states (a–c) were reconstructed using the PGLS method (Martins and Hansen, 1997).

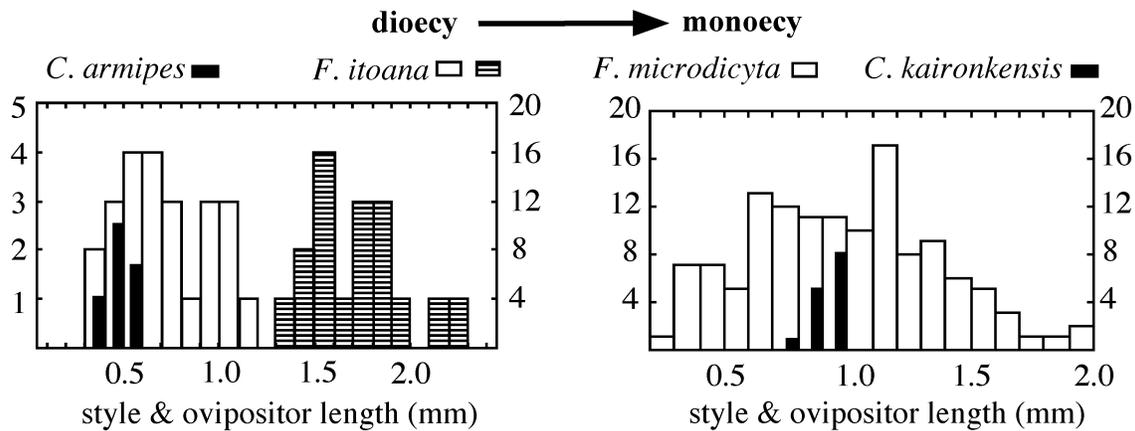


FIGURE 5. Sister-group comparison of dioecious *Ficus itoana* and monoecious *F. microdictya* and their pollinators, an unambiguous cospeciation event (Weiblen and Bush, 2002). Frequency distributions of style lengths (open and hatched bars) and ovipositor lengths (solid bars) are compared for this apparent reversal to monoecy in a dioecious clade (Weiblen, 2000). Hatched bars represent style lengths in seed figs. The frequencies of pollinator and fig measures are scaled at the left and right of each plot, respectively.

monoecious fig pollinators approximate the mean style length of their host figs, whereas ovipositors in dioecious fig pollinators match the length of styles in gall figs (open bars in Fig. 5). Accordingly, subsequent comparisons of style and ovipositor lengths were limited to gall figs in functionally dioecious species.

Trait measurements for figs, pollinators, and nonpollinators, including ovipositor length, are summarized in Table 2. In spite of small sample sizes for many species, most interacting trait correlations were significant. Ahistorical correlations, independent contrasts, and residuals from phylogenetic autocorrelation showed that consideration of phylogeny affected the strength but not the direction of trait interrelationships (Table 3). For independent contrasts, whether a fig or pollinator molecular phylogeny was assumed did not influence the strength of correlation. For phylogenetic autoregression, results were also similar for phylogenies based on molecular data alone or based on the combination of molecular and morphological data.

Gall width showed a significant relationship with fig wall width and style length, and ovipositor length was significantly correlated with thorax length in pollinators and nonpollinators. The most compelling evidence for interspecific coevolution was that interacting trait associations between lineages (e.g., fig styles and pollinator ovipositors) were stronger than associations in the same lineage (e.g., fig wall thickness and style length).

Correlation of fig style length and pollinator ovipositor length (Fig. 6) probably was not due to fig breeding system variation, given that the correlation remained strong in analyses of monoecious species alone (Murray, 1985; Ganeshiah et al., 1995; Nefdt and Compton, 1996). A pair of monoecious and functionally dioecious clades could yield a spurious correlation between style and ovipositor length arising from the clustering of two sets of nonindependent data points (Felsenstein, 1985; Harvey and Pagel, 1991). However, these functional groups are not monophyletic, and style

and pollinator ovipositor length remained highly correlated even after phylogenetic effects were considered (Fig. 6).

There were also significant relationships between gall size and body size for pollinating and nonpollinating wasps (Fig. 6). The relationship was somewhat weaker for nonpollinators, where the allometry of extremely long ovipositors could influence body size (Compton and Nefdt, 1988). Nonpollinating *Sycoscapter* ovipositor length was highly correlated with the thickness of the fig wall, which is pierced from the exterior to reach fig flowers on the interior. By contrast, there was no relationship between wall thickness and ovipositor length in pollinators who instead pierce the stigmatic surface from within the fig (Table 3).

## DISCUSSION

### *Correlated Evolution and Coadaptation*

The application of comparative methods (Harvey and Pagel, 1991) has increased as the importance of considering the phylogenetic relationships of interspecific data has gained acceptance among evolutionary biologists interested in adaptation (Coddington, 1988; Baum and Larson, 1991; Frumhoff and Reeve, 1994; Leroi et al., 1994; Losos and Miles, 1994). This is one of the first studies incorporating two molecular phylogenies in a comparative analysis of coevolution and includes the largest taxonomic sample ever analyzed. Phylogenetic analysis established that particular pollinator and fig traits have each evolved a number of times and that evolutionary changes in closely interacting traits are strongly correlated. When pollinator ovipositors were reconstructed on a fig phylogeny, for example, reduction and elongation in this trait was associated with the gain and loss of functional dioecy (Fig. 4). The same was true when fig traits were reconstructed on a pollinator phylogeny. A twin-phylogeny approach evaluates hypotheses of coadaptation for interacting lineages in a reciprocal manner, as

TABLE 2. Fig, pollinator, and nonpollinator trait values for comparative analyses of correlated evolution. Associations are numbered as in Table 1. Only style lengths in gall figs are listed, owing to the bimodal distribution of the trait in functionally dioecious species (Weiblen et al., 2001).

Association	Host fig						Pollinator			Nonpollinator		
	Wall width (cm)		Style length (mm)		Gall width (mm)		Ovip length (mm)	Thorax length (mm)		Ovip length (mm)	Thorax length (mm)	
	$\bar{x}$ (SE)	N	$\bar{x}$ (SE)	N	$\bar{x}$ (SE)	N	$\bar{x}$ (SE)	$\bar{x}$ (SE)	N	$\bar{x}$ (SE)	$\bar{x}$ (SE)	N
1	0.39 (0.03)	3	0.40 (0.07)	5	1.10 (0.03)	5	0.05	0.54	1	2.72 (0.09)	0.41 (0.00)	3
2	0.12 (0.02)	4	0.40 (0.04)	5	1.14 (0.05)	5	0.12 (0.02)	0.54 (0.01)	5	3.26 (0.27)	0.48 (0.02)	5
3	0.40 (0.02)	43	0.30 (0.02)	10	0.99 (0.02)	5	0.34 (0.01)	0.57 (0.04)	5	3.54 (0.06)	0.45 (0.03)	5
4	0.29 (0.01)	36	0.42 (0.01)	5	1.03 (0.02)	5	0.17 (0.01)	0.56 (0.03)	5	4.17 (0.17)	0.47 (0.05)	4
5	0.40 (0.16)	2	1.25 (0.07)	2	1.70 (0.03)	2	1.01 (0.02)	0.69 (0.02)	5			
6	0.39 (0.00)	2	1.75 (0.02)	99	1.63 (0.03)	3	1.17 (0.05)	0.64 (0.01)	5			
7	0.57 (0.02)	3	0.50 (0.01)	5	0.95 (0.05)	5	0.50 (0.02)	0.54 (0.02)	5			
8	0.70 (0.08)	3	0.39 (0.07)	4	1.20 (0.03)	4	0.37 (0.02)	0.69 (0.03)	5			
9	0.39 (0.00)	6	1.02 (0.07)	61	1.32 (0.08)	5	0.99 (0.00)	0.62 (0.01)	5			
10	0.57 (0.01)	3	0.66 (0.04)	6	1.21 (0.03)	6	0.33 (0.02)	0.63 (0.04)	5	8.44 (0.25)	0.70 (0.03)	5
11	0.14 (0.00)	10	0.42 (0.01)	5	0.87 (0.02)	5	0.24 (0.02)	0.52 (0.02)	5			
12	0.01 (0.00)	35	0.39 (0.02)	48	0.77 (0.01)	10	0.25 (0.02)	0.43 (0.01)	33	2.95 (0.02)	0.41 (0.01)	3
13	0.45 (0.01)	35	0.54 (0.01)	59	1.19 (0.01)	5	0.58 (0.02)	0.54 (0.02)	59	7.16 (0.17)	0.60 (0.06)	5
14	0.43 (0.01)	3	0.50 (0.03)	5	1.06 (0.12)	5	0.31 (0.02)	0.49 (0.02)	3			
15	0.37 (0.02)	33	0.50 (0.05)	5	0.96 (0.05)	5	0.26 (0.03)	0.59 (0.01)	5	7.23 (0.13)	0.55 (0.02)	2
16	0.68 (0.02)	66	0.35 (0.02)	180	1.28 (0.04)	5	0.25 (0.00)	0.75 (0.02)	5	10.93 (0.33)	0.69 (0.03)	5
17	0.12 (0.03)	17	0.37 (0.02)	5	1.05 (0.03)	5	0.19 (0.01)	0.68 (0.05)	5	5.69 (0.20)	0.68 (0.02)	3
18	0.87 (0.05)	17	1.24 (0.04)	5	1.82 (0.07)	5	0.59 (0.04)	1.10 (0.03)	5	15.25 (0.46)	0.94 (0.01)	5
19	0.85 (0.01)	5	0.82 (0.05)	25	1.38 (0.04)	5	0.46 (0.02)	0.95 (0.01)	20			
20	0.31 (0.02)	3	0.98 (0.03)	129	1.29 (0.03)	10	0.89 (0.01)	0.65 (0.02)	14	5.91 (0.41)	0.52 (0.05)	3
21	0.29 (0.02)	3	0.59 (0.04)	6	1.60 (0.07)	5	0.27 (0.01)	1.00 (0.02)	5			
22	0.41 (0.06)	3	1.76 (0.20)	12	1.25 (0.04)	5	1.20 (0.05)	0.64 (0.04)	5			
23	0.17 (0.01)	3	1.32 (0.13)	12	0.74 (0.03)	5	0.68 (0.03)	0.41 (0.01)	5			
24	0.12 (0.01)	3	1.11 (0.10)	12	0.69 (0.01)	5	0.67 (0.04)	0.60 (0.01)	3			
25	0.06 (0.01)	12	0.99 (0.10)	12	0.53 (0.03)	5	0.83 (0.06)	0.40 (0.01)	5	3.19 (0.04)	0.44 (0.01)	5
26	0.54 (0.00)	32	0.65 (0.05)	5	1.18 (0.03)	5	0.56 (0.03)	0.74 (0.02)	5	10.63 (0.23)	0.82 (0.03)	5
27	0.39 (0.00)	25	0.70 (0.06)	5	1.45 (0.05)	5	0.21 (0.02)	0.78 (0.04)	5	8.23 (0.29)	0.97 (0.01)	5
28	0.04 (0.00)	25	0.40 (0.05)	5	0.96 (0.05)	5	0.29 (0.02)	0.41 (0.02)	5	5.08 (0.06)	0.52 (0.01)	4
29	0.02 (0.01)	25	0.41 (0.06)	6	0.62 (0.01)	5	0.18 (0.01)	0.39 (0.02)	5	1.78 (0.02)	0.48 (0.02)	5
30	0.12 (0.01)	31	0.47 (0.04)	5	0.91 (0.02)	5	0.29 (0.03)	0.55 (0.02)	5	5.49 (0.08)	0.57 (0.03)	4
31	0.12 (0.00)	24	0.36 (0.02)	5	0.81 (0.03)	5	0.14 (0.01)	0.49 (0.02)	5	3.41 (0.07)	0.53 (0.03)	5
32	0.15 (0.01)	4	0.33 (0.06)	5	0.68 (0.05)	5	0.17 (0.01)	0.37 (0.02)	5	2.73 (0.15)	0.44 (0.03)	4
33	0.14 (0.11)	6	1.13 (0.13)	11	0.95 (0.04)	5	0.74 (0.02)	0.48 (0.02)	5			
34	0.01 (0.06)	3	0.90 (0.09)	18	0.70 (0.02)	5	0.56 (0.01)	0.36 (0.02)	5			
35	0.48 (0.00)	3	1.32 (0.20)	11	1.98 (0.05)	5	1.14 (0.07)	1.41 (0.06)	5	7.46 (0.23)	0.66 (0.03)	5
36	0.14 (0.01)	5	1.29 (0.17)	14	0.86 (0.05)	5	0.70 (0.01)	0.86 (0.06)	5	4.71 (0.17)	0.68 (0.01)	5
37	0.22 (0.02)	4	0.92 (0.11)	12	0.96 (0.02)	5	0.34 (0.02)	0.55 (0.05)	5	2.80 (0.25)	0.58 (0.04)	3
38	0.75 (0.02)	3	1.94 (0.24)	11	1.37 (0.10)	3	1.15 (0.13)	0.76 (0.04)	3			
39	0.13 (0.00)	3	1.56 (0.11)	11	1.16 (0.04)	6	1.12 (0.03)	0.65 (0.01)	5	5.74 (0.12)	0.68 (0.02)	5
40	1.09 (0.28)	3	0.71 (0.05)	12	1.30 (0.03)	7	0.36 (0.04)	0.69 (0.01)	2			
41	0.30 (0.02)	3	0.47 (0.05)	5	0.97 (0.04)	5	0.18 (0.02)	0.48 (0.02)	5			
42	0.47 (0.01)	3	0.80 (0.04)	5	1.19 (0.04)	5	0.24 (0.01)	0.55 (0.03)	5			

opposed to limiting consideration to cospeciating groups (Morand et al., 2000).

Coadaptation is a special case of adaptation in which correlated evolution is predicted even though the interacting traits may not share the same phylogenetic history. In fig pollination mutualism, for example, ancestral host switching may have brought pollinators into novel associations with hosts, resulting in the reciprocal change of closely interacting traits such as style and ovipositor lengths. How can correlated evolution be evaluated when the histories of the traits in question are not identical? One approach is to calculate a set of contrasts on each phylogeny and to reverse the dependence of the variables when computing their correlation. This technique is not entirely satisfactory, however, because the history of the independent variable may differ from that

of the dependent variable (Morand et al., 2000). Phylogenetic autocorrelation solves this problem by partitioning the variance in trait values of terminal taxa into residual and phylogenetic components, for which two traits need not share the same history. What remains after the phylogenetic components have been stripped from each trait is the correlation due to factors other than phylogenetic inertia. However, the evolutionary interpretation of such correlations is unclear because coadaptation is likely to have occurred along the diversifying branches of interacting phylogenies and not merely at their tips.

Some additional problems associated with phylogenetic tests of correlated evolution include limited numbers of evolutionary events (Maddison, 1990), insufficient taxon sampling (Sillen-Tullberg, 1993), phylogenetic accuracy (Donoghue and Ackerly, 1996), and

TABLE 3. Correlated evolution of continuous morphological traits in figs and fig wasps. A historical correlations (AC) are Pearson's product-moment correlations between dependent traits ( $x$ ) and independent traits ( $y$ ). Independent contrast correlations (IC) are reported for fig and fig wasp molecular phylogenies separately. Residual correlations (RC) for interacting traits were derived from separate phylogenetic autoregression models for fig and fig wasp phylogenies. ML branch lengths were calculated for most-parsimonious trees derived from analyses of molecular data alone and from analysis of the combination of molecular and morphological data. Nonpollinator trait correlations were calculated using fig and pollinator topologies pruned to include only those species associated with *Sycoscapter*. ovip = ovipositor; pol = pollinator; npol = nonpollinator; ln = length; wd = width.

$x$	$y$	AC	IC		RC	
			Fig	Pollinator	Molecular	Combined
Within-lineage correlations						
style ln	wall wd	0.197	0.317		0.259	0.265
gall wd	wall wd	0.604 <sup>a</sup>	0.613 <sup>a</sup>		0.600 <sup>a</sup>	0.569 <sup>a</sup>
style ln	gall wd	0.403 <sup>b</sup>	0.458 <sup>b</sup>		0.421 <sup>b</sup>	0.437 <sup>b</sup>
pol ovip ln	pol thorax ln	0.316 <sup>c</sup>		0.306	0.320 <sup>c</sup>	0.319 <sup>c</sup>
npol ovip ln	npol thorax ln	0.831 <sup>a</sup>		0.809 <sup>a</sup>	0.830 <sup>a</sup>	0.830 <sup>a</sup>
Among-lineage correlations						
Mutualism						
style ln	pol ovip ln	0.880 <sup>a</sup>	0.839 <sup>a</sup>	0.761 <sup>a</sup>	0.861 <sup>a</sup>	0.818 <sup>a</sup>
wall wd	pol ovip ln	0.123	0.334 <sup>c</sup>	0.053	0.116	0.126
gall wd	pol thorax ln	0.795 <sup>a</sup>	0.824 <sup>a</sup>	0.784 <sup>a</sup>	0.796 <sup>a</sup>	0.768 <sup>a</sup>
Parasitism						
style ln	npol ovip ln	0.433 <sup>c</sup>	0.609 <sup>c</sup>	0.613 <sup>b</sup>	0.446 <sup>c</sup>	0.446 <sup>c</sup>
wall wd	npol ovip ln	0.811 <sup>a</sup>	0.851 <sup>a</sup>	0.851 <sup>a</sup>	0.844 <sup>a</sup>	0.844 <sup>a</sup>
gall wd	npol thorax ln	0.633 <sup>b</sup>	0.676 <sup>b</sup>	0.687 <sup>b</sup>	0.628 <sup>b</sup>	0.628 <sup>b</sup>

<sup>a</sup>  $p < 0.0001$ .

<sup>b</sup>  $p < 0.01$ .

<sup>c</sup>  $p < 0.05$ .

uncertainty associated with the reconstruction of ancestral states (Cunningham et al., 1998). For example, using parsimony and ML methods for discrete characters, I was unable to determine which changed first, fig breeding system or pollinator ovipositor length, because the changes were coincident. However, phylogenetic reconstruction of continuous characters was sufficient to evaluate several hypotheses of adaptive evolution statistically. Independent contrasts and phylogenetic autocorrelation yielded highly similar results, and tests typically were not sensitive to the choice of tree topology. All available methods indicated that between-lineage interacting trait correlations are stronger than the allometric relationships of traits within lineages (Table 3). For example, fig style lengths were more highly correlated with pollinator ovipositor lengths than with the size of fig flowers. Also as predicted, noninteracting traits (e.g., pollinator ovipositors and the fig wall) were not as strongly correlated as were traits within these lineages (e.g., fig wall and flower size).

The role that traits such as style length and ovipositor length have played in the maintenance of fig/pollinator mutualism remains controversial (Herre and West, 1997; Herre et al., 1999). Nonetheless, a strong evolutionary correlation between these traits is evidence that pollinator ovipositors have responded rapidly to evolutionary changes in the distribution of style lengths, and vice versa. Ancestral changes in these traits were so closely coincident that it was not possible to infer the sequence of change. The problem of disentangling cause and effect is not unique to studies of adaptation, but patterns of correlated evolution are at least consistent with the predictions of fitness models (Kiestler et al., 1984; Ganeshaiah et al., 1995).

Correlated change in styles and ovipositors (Figs. 4–6) might result from style length distributions selecting for an optimal ovipositor length (Ganeshaiah et al., 1995) and the fitness cost of ovule-galling pollinators selecting for an optimal style length distribution (Kiestler et al., 1984). Style length variance exceeds what is needed for optimal packing of flowers inside of figs (Ganeshaiah and Kathuria, 1999), and high variance in style length compared with that in ovipositor length suggests either diversifying selection on style length or stabilizing selection on ovipositor length (Table 2). As in any co-evolutionary scenario, these processes are not mutually exclusive.

Evolutionary changes in pollinator ovipositor length were also closely coupled with changes in fig breeding system between monoecy and dioecy. The ovipositors of dioecious fig pollinators were highly correlated with the mean style length of hospitable gall figs and were consistently shorter than the style lengths of pollinators of inhospitable seed figs (Fig. 5). The inability of pollinators to distinguish between gall and seed figs prior to entering the "tomb blossom" (Patel et al., 1995; Anstett et al., 1998) has been attributed to vicarious selection (Graff and Godfray, 1991), which refers to the increased plant fitness associated with rearing the offspring of visitors to gall figs that are unable to avoid seed figs. Low intraspecific ovipositor length variation (Table 2) and the tight correlation with gall fig style length suggest that ovipositor length in dioecious fig pollinators has evolved in response to gall figs as opposed to seed figs.

Correlated evolution is further evident in the relationship between ovipositor length and fig wasp life history, whether mutualistic or parasitic. Ovipositor length in nonpollinators interacting with the fig wall was

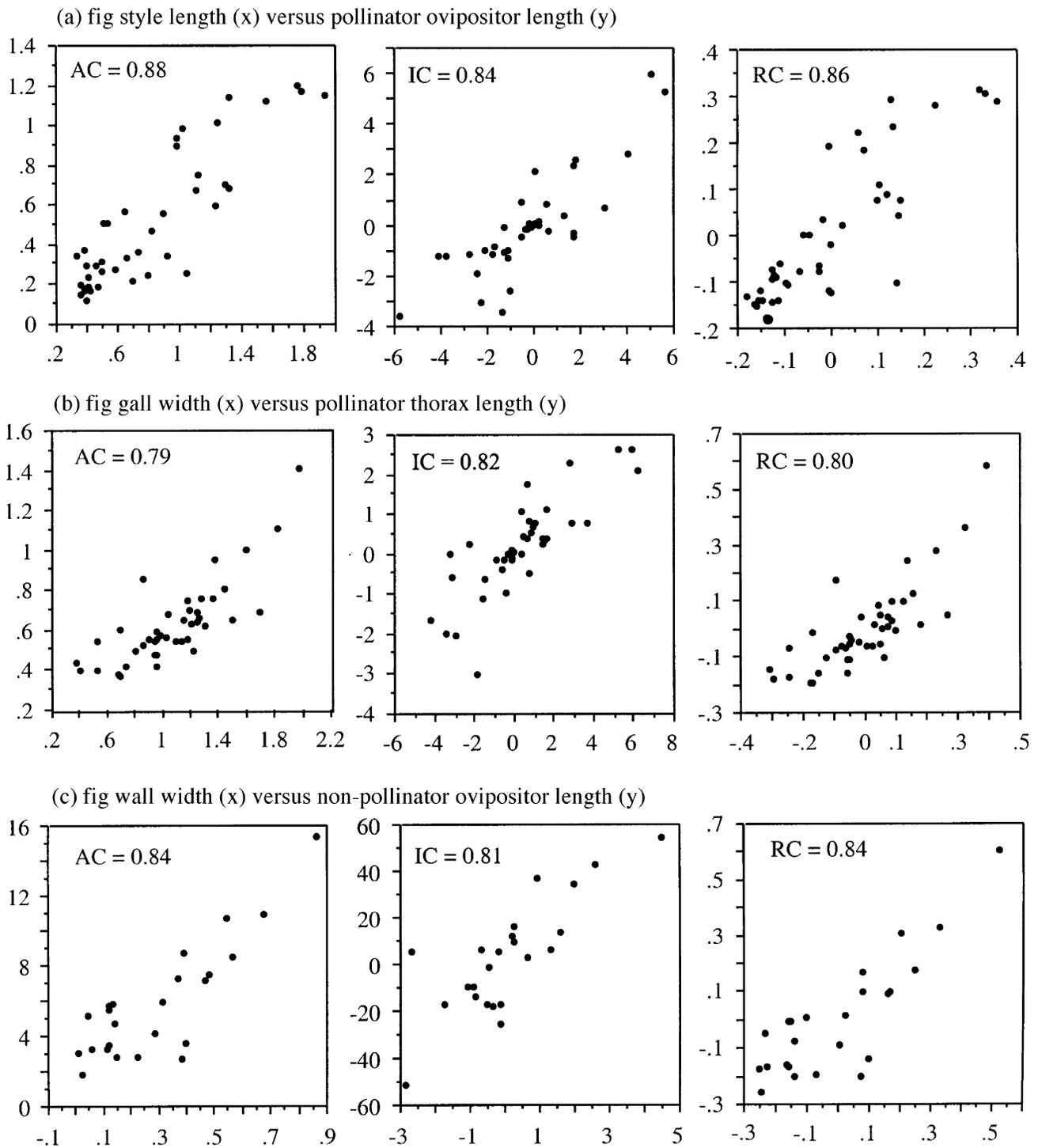


FIGURE 6. Correlated evolution of traits in figs, pollinators, and nonpollinators. (a) Style length versus pollinator ovipositor length. (b) Gall width versus pollinator thorax length. (c) Fig wall width versus nonpollinator ovipositor length. Units are millimeters. AC = coefficients of ahistorical correlations; IC = independent contrasts; RC = residual correlations. Independent contrasts are shown with respect to fig phylogeny. Fig and wasp trait residuals were calculated against their respective molecular phylogenies.

correlated with wall thickness to a much greater extent than it was with style length. Conversely, pollinator ovipositor length was highly correlated with style length but not with the thickness of the fig wall.

Mutualism and parasitism thus provide an ecological contrast, demonstrating that correlations between interacting traits are significantly stronger than the associations between traits that do not interact.

Correlations between interacting traits (e.g., nonpollinator ovipositor length and the fig wall) were stronger than allometric correlations within lineages (e.g., fig wall thickness and gall width), which suggests that interactions between lineages might lead to the decoupling of developmental correlations within lineages. Could hypotheses of coadaptation be evaluated by testing for the disruption of allometric relationships within lineages in favor of trait correlations between interacting lineages? Although such evidence of correlated evolution does not establish causation, it may help to identify experimental tests of particular coadaptation hypotheses. For example, the correlated evolution of wasp body size and fig gall size supports the role that resource limitation might play in mutualism stability. Herre (1989) proposed that gall size could constrain pollinator egg load and contribute to the regulation of seed and pollinator production. Experiments on patterns of egg deposition in fig flowers could test the functional roles of these correlated traits in mitigating evolutionary conflicts between figs and pollinators (Jousselin et al., 2001).

#### CONCLUSIONS

Examples from fig pollination illustrate how a comparative phylogenetic approach can evaluate hypotheses of interspecific correlated evolution. Methods developed for testing the significance of association between two traits within a single lineage can be extended to the special case of interacting lineages, where traits may not share a common phylogenetic history. Reciprocal tests based on alternative phylogenies can be conducted, and the dependence of the variables can be reversed in each case. For example, when mapping traits of associates on a host phylogeny, the traits are treated as an independent feature of the environment. Methods of independent contrasts and phylogenetic autocorrelation including a single history or two separate histories yield similar results but differ in their interpretation. The application of these techniques to coevolutionary problems outlines a new direction for the development of comparative methods. In particular, analysis of coadaptation could be advanced with methods for detecting the displacement of within-lineage correlations by trait correlations between lineages.

#### ACKNOWLEDGMENTS

I thank D. Adams, D. Baum, J. Cook, M. Donoghue, C. Favret, T. Garland, K. Johnson, C. Lopez-Vaamonde, E. Kellogg, F. Kjellberg, A. Herre, E. Jousselin, J. Losos, C. Machado, M. Pagel, and D. Yu for helpful comments and suggestions. This research was funded by National Science Foundation dissertation improvement grant DEB-9801259.

#### REFERENCES

- Anstett, M. C., M. Gibernau, and M. Hossaert-McKey. 1998. Partial avoidance of female inflorescences of a dioecious fig by their mutualistic pollinating wasps. *Proc. R. Soc. Lond. B* 265:45–50.
- Anstett, M. C., M. Hossaert-McKey, and F. Kjellberg. 1997. Figs and fig pollinators: Evolutionary conflicts in a coevolved mutualism. *Trends Ecol. Evol.* 12:94–98.
- Arnqvist, G., and L. Rowe. 2002. Correlated evolution of male and female morphologies in water striders. *Evolution* 56:936–947.
- Baijnath, H., and S. Ramcharun. 1983. Aspects of pollination and floral development in *Ficus capensis* Thunb. (Moraceae). *Bothalia* 14:883–888.
- Baum, D. A., and A. Larson. 1991. Adaptation reviewed: A phylogenetic methodology for studying character macroevolution. *Syst. Zool.* 40:1–18.
- Berg, C. C. 1986. The *Ficus* species (Moraceae) of Madagascar and the Comoro Islands. *Bull. Mus. Nat. Hist. Nat. Paris* 8:17–55.
- Berg, C. C. 1990. Reproduction and evolution of *Ficus* (Moraceae): Traits connected with the adequate rearing of pollinators. *Mem. N.Y. Bot. Gard.* 55:169–185.
- Bronstein, J. L. 1988. Mutualism, antagonism and the fig-pollinator interaction. *Ecology* 69:1298–1302.
- Clayton, D. H., S. Al-Tamimi, and K. P. Johnson. 2003. The ecological basis of coevolutionary history. Pages 310–350 *in* *Tangled trees: Phylogeny, speciation, and coevolution* (R. D. M. Page, ed.). Univ. Chicago Press, Chicago.
- Coddington, J. A. 1988. Cladistic tests of adaptational hypotheses. *Cladistics* 4:3–22.
- Compton, S. G., and R. J. C. Nefdt. 1988. Extra-long ovipositors in chalcid wasps: Some examples and observations. *Antenna* 12:102–105.
- Corner, E. J. H. 1985. *Ficus* (Moraceae) and Hymenoptera (Chalcidoidea): Figs and their pollinators. *Biol. J. Linn. Soc.* 25:187–195.
- Cunningham, C. W., K. E. Omland, and T. H. Oakley. 1998. Reconstructing ancestral character states: A critical reappraisal. *Trends Ecol. Evol.* 13:361–366.
- Cunningham, D. D. 1888. On the phenomena of fertilization in *Ficus roxburghii* Wall. *Ann. R. Bot. Gard. Calcutta* 1(Appendix):11–51.
- Donoghue, M. J. 1989. Phylogenies and the analysis of evolutionary sequences with examples from the seed plants. *Evolution* 43:1137–1156.
- Donoghue, M. J., and D. A. Ackerly. 1996. Phylogenetic uncertainties and sensitivity analyses in comparative biology. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 351:1241–1249.
- Felsenstein, J. 1985. Phylogenies and the comparative method. *Am. Nat.* 125:1–15.
- Frumhoff, P. C., and H. K. Reeve. 1994. Using phylogenies to test hypotheses of adaptation: A critique of some current proposals. *Evolution* 48:172–180.
- Futuyma, D. J., and M. Slatkin (eds.). 1983. *Coevolution*. Sinauer, Sunderland, Massachusetts.
- Ganeshiah, K. N., and P. Kathuria. 1999. Does optimal packing of flowers in syconia shape style length variation in monoecious figs? *Biotropica* 31:312–320.
- Ganeshiah, K. N., P. Kathuria, R. U. Shaanker, and R. Vasudeva. 1995. Evolution of style-length variability in figs and optimization of ovipositor length in their pollinator wasps: A coevolutionary model. *J. Genet.* 74:25–39.
- Garland, T., P. H. Harvey, and A. R. Ives. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst. Biol.* 41:18–32.
- Grafen, A., and H. C. J. Godfray. 1991. Vicarious selection explains some paradoxes in dioecious fig–pollinator systems. *Proc. R. Soc. Lond. B* 245:73–76.
- Harrison, R. D., and N. Yamamura. 2003. A few more hypotheses for the evolution of dioecy in figs (*Ficus*, Moraceae). *Oikos* 100:628–635.
- Harvey, P. H., and M. D. Pagel. 1991. *The comparative method in evolutionary biology*. Oxford Univ. Press, Oxford, UK.
- Herre, E. A. 1989. Coevolution of reproductive characteristics in 12 species of New World figs and their pollinator wasps. *Experientia* 45:637–647.
- Herre, E. A., N. Knowlton, U. G. Mueller, and S. A. Rehner. 1999. The evolution of mutualisms: Exploring the paths between conflict and cooperation. *Trends Ecol. Evol.* 14:49–53.
- Herre, E. A., C. A. Machado, E. Bermingham, J. D. Nason, D. M. Windsor, S. S. McCafferty, W. V. Houten, and K. Bachmann. 1996. Molecular phylogenies of figs and their pollinator wasps. *J. Biogeogr.* 23:521–530.

- Herre, E. A., and S. A. West. 1997. Conflict of interest in a mutualism: Documenting the elusive fig-wasp-seed tradeoff. *Proc. R. Soc. Lond. B* 264:1501–1507.
- Janzen, D. H. 1979. How to be a fig. *Annu. Rev. Ecol. Syst.* 10:13–51.
- Jousselin, E., M. Hossaert-McKey, D. Vernet, and F. Kjellberg. 2001. Egg deposition patterns of fig pollinating wasps: Implications for studies on the stability of the mutualism. *Ecol. Entomol.* 26:602–608.
- Kathuria, P., K. N. Ganeshiah, R. U. Shaanker, and R. Vasudeva. 1995. Is there a dimorphism for style lengths in monoecious figs? *Curr. Sci.* 68:1047–1050.
- Kerdelhué, C., and J.-Y. Rasplus. 1996. The evolution of dioecy among *Ficus* (Moraceae): An alternate hypothesis involving non-pollinating fig wasp pressure on the fig-pollinator mutualism. *Oikos* 77:163–166.
- Kiester, A. R., R. Lande, and D. W. Schemske. 1984. Models of coevolution and speciation in plants and their pollinators. *Am. Nat.* 124:220–243.
- Kjellberg, F., P. H. Gouyon, M. Ibrahim, M. Raymond, and G. Valdeyron. 1987. The stability of the symbiosis between dioecious figs and their pollinators: A study of *Ficus carica* L. and *Blastophaga psenes* L. *Evolution* 41:693–704.
- Laman, T. G., and G. D. Weiblen. 1998. Figs of Gunung Palung National Park (West Kalimantan, Indonesia). *Trop. Biodiv.* 5:245–297.
- Leroi, A. M., M. R. Rose, and G. V. Lauder. 1994. What does the comparative method reveal about adaptation? *Am. Nat.* 143:381–402.
- Lopez-Vaamonde, C., J.-Y. Rasplus, G. Weiblen, and J. M. Cook. 2001. DNA-based phylogenies of fig wasps: Partial co-cladogenesis between pollinators and parasites. *Mol. Phylogenet. Evol.* 21:55–71.
- Losos, J. B., and D. B. Miles. 1994. Adaptation, constraint, and the comparative method: Phylogenetic issues and methods. Pages 60–98 in *Ecological morphology* (P. C. Wainwright and S. M. Reilly, eds.). Univ. Chicago Press, Chicago.
- Machado, C. A., E. A. Herre, S. McCafferty, and E. Birmingham. 1996. Molecular phylogenies of fig pollinating and nonpollinating wasps and the implications for the origin and evolution of the fig-fig wasp mutualism. *J. Biogeogr.* 23:531–542.
- Machado, C. A., E. Jousselin, F. Kjellberg, S. G. Compton, and E. A. Herre. 2001. Phylogenetic relationships, historical biogeography and character evolution of fig-pollinating wasps. *Proc. R. Soc. Lond. B* 268:685–694.
- Maddison, W. P. 1990. A method for testing the correlated evolution of two binary characters: Are gains or losses concentrated on certain branches of a phylogenetic tree? *Evolution* 44:539–557.
- Maddison, W. P., and D. R. Maddison. 1992. *MacClade: Analysis of phylogeny and character evolution*, version 3. Sinauer, Sunderland, Massachusetts.
- Martins, E. P. 2000. Adaptation and the comparative method. *Trends Ecol. Evol.* 15:296–298.
- Martins, E. P. 2001. COMPARE 4.4. Computer programs for the statistical analysis of comparative data. Department of Biology, Indiana Univ., Bloomington.
- Martins, E. P., and T. Garland. 1991. Phylogenetic analyses of the correlated evolution of continuous characters: A simulation study. *Evolution* 45:534–557.
- Martins, E. P., and T. F. Hansen. 1997. Phylogenies and the comparative method: A general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am. Nat.* 149:646–667.
- Morand, S., M. S. Hafner, R. D. M. Page, and D. L. Reed. 2000. Comparative body size relationships in pocket gophers and their chewing lice. *Biol. J. Linn. Soc.* 70:239–249.
- Murray, M. G. 1985. Figs (*Ficus* spp.) and fig wasps (Chalcidoidea, Agaonidae): Hypotheses for an ancient symbiosis. *Biol. J. Linn. Soc.* 26:69–81.
- Nefdt, R. J. C., and S. G. Compton. 1996. Regulation of seed and pollinator production in the fig-fig wasp mutualism. *J. Anim. Ecol.* 65:170–182.
- Page, R. D. M. (ed.). 2003. *Tangled trees: Phylogeny, Cospeciation, and Coevolution*. Univ. Chicago Press, Chicago.
- Pagel, M. 1994. Detecting correlated evolution on phylogenies: A general method for the comparative analysis of discrete characters. *Proc. R. Soc. Lond. B* 255:37–45.
- Pagel, M. 1997. Inferring evolutionary processes from phylogenies. *Zool. Scr.* 26:331–348.
- Patel, A., M. Anstett, M. Hossaert-McKey, and F. Kjellberg. 1995. Pollinators entering female dioecious figs: Why commit suicide? *J. Evol. Biol.* 8:301–313.
- Ramirez, W. B. 1978. Evolution of mechanisms to carry pollen in Agaonidae (Hymenoptera Chalcidoidea). *Tijdschr. Entomol.* 121:279–293.
- Ramirez, W. B. 1980. Evolution of the monoecious and dioecious habit in *Ficus* (Moraceae). *Brenesia* 18:207–215.
- Sillen-Tullberg, B. 1993. The effect of biased inclusion of taxa on the correlation between discrete characters in the phylogenetic trees. *Evolution* 47:1182–1191.
- van Noort, S., and S. G. Compton. 1996. Convergent evolution of agaonine and sycoecine (Agaonidae, Chalcidoidea) head shape in response to the constraints of host fig morphology. *J. Biogeogr.* 23:415–424.
- Verkerke, W. 1988. Sycone morphology and its influence on the flower structure of *Ficus sur* (Moraceae). *Proc. K. Ned. Akad. Wet. Ser. C Biol. Med. Sci.* 91:321–344.
- Weiblen, G. D. 1999. Phylogeny and ecology of dioecious fig pollination. Ph.D. Thesis, Harvard Univ., Cambridge, Massachusetts.
- Weiblen, G. D. 2000. Phylogenetic relationships of functionally dioecious *Ficus* (Moraceae) based on ribosomal DNA sequences and morphology. *Am. J. Bot.* 87:1342–1357.
- Weiblen, G. D. 2001. Phylogenetic relationships of fig wasps pollinating functionally dioecious figs based on mitochondrial DNA sequences and morphology. *Syst. Biol.* 50:243–267.
- Weiblen, G. D., and G. L. Bush. 2002. Speciation in fig pollinators and parasites. *Mol. Ecol.* 11:1573–1578.
- Weiblen, G., B. Flick, and H. Spencer. 1995. Seed set and wasp predation in dioecious *Ficus variegata* from an Australian wet tropical forest. *Biotropica* 27:391–394.
- Weiblen, G. D., D. W. Yu, and S. A. West. 2001. Pollination and parasitism in functionally dioecious figs. *Proc. R. Soc. Lond. B* 268:651–659.
- Wiebes, J. T. 1963. Taxonomy and host preferences of Indo-Australian fig wasps of the genus *Ceratosolen* (Agaonidae). *Tijdschr. Entomol.* 106:1–112.
- Wiebes, J. T. 1979. Co-evolution of figs and their insect pollinators. *Annu. Rev. Ecol. Syst.* 10:1–12.
- Wiebes, J. T. 1981. The species-group of *Ceratosolen armipes* Wiebes (Hymenoptera Chalcidoidea, Agaonidae). *Proc. K. Ned. Akad. Wet. Ser. C Biol. Med. Sci.* 84:365–377.
- Yokoyama, J. 1995. Insect-plant coevolution and speciation. Pages 115–130 in *Biodiversity and evolution* (R. Arai, M. Kato, and Y. Doi, eds.). National Science Museum Foundation, Tokyo.

Final acceptance 14 September 2003

Associate Editor: Kevin Johnson