CHAPTER 7

Coevolution in mutualisms

Bruce Anderson

7.1 Introduction

Coevolution can be defined as the reciprocal evolutionary change of interacting species, driven by natural selection. A frequently cited example of coevolution stems from the early writings of Darwin (1862): Having examined the extraordinarily long nectar spurs of the Madagascan star orchid *Angraecum sesquipedale*, Darwin successfully predicted the existence of a seemingly implausible hawkmoth pollinator with a tongue measuring close to 30 cm in length. In explaining his prediction, Darwin described the first mechanistic model of the coevolutionary process, hypothesizing that selection induced by the orchid's long nectar spurs drove the evolution of even longer moth tongues, enabling the moths to access nectar deeper within the flower. Simultaneously, he posited that the evolution of longer moth tongues exerted selection for longer nectar spurs so that pollen transfer would be more efficient, resulting in "a race in gaining length" between moth tongues and floral nectaries (Darwin 1862). Darwin's prediction was confirmed some forty years later when a hawkmoth with an extremely long proboscis was found in Madagascar.

However, as Arditti et al. (2012) point out, coevolution is not confirmed by the mere interaction between a moth with a long proboscis and a flower with a long spur. Long spurs and proboscides could potentially be adaptations to other unrelated selective pressures. For example, it has been proposed that the long moth proboscides did not evolve to sip nectar from long-tubed flowers, but rather to distance the moths from the attractive parts of short-tubed flowers where predatory spiders lie in wait

(Wasserthall 1997). Such debates speak to the historical controversy surrounding the coevolutionary concept, and the consequent oscillations between general acceptance of coevolution as a powerful selective force, and general scepticism of its importance. Perhaps one problem with the hawkmoth–orchid exemplar is that it gives the impression that reciprocal selection can only be very narrowly applied to two reciprocally interacting traits such as proboscides and spurs, involved in an apparently positive feedback cycle. However, coevolution can also be used more broadly to encompass situations where two organisms (rather than just two interacting traits) undergo some form of evolutionary change or adaptive response to one another (as used by Thompson 1994, 2005). Importantly, adaptations should only be defined as coevolutionary when they are a result of a shared evolutionary history, not simply a chance conjoining of two species with preadapted or preexisting complimentary traits (also see Section 3.3.1 for what constitutes adaptation).

In this chapter, I will provide a brief history of coevolutionary studies, demonstrating that the concept can be equally applied to mutualisms and antagonistic relationships. Next I shall discuss some of the challenges in demonstrating coevolution, highlighting studies that provide powerful evidence for reciprocal selection and evolutionary change. Following this, I review some of the patterns associated with phenotypic traits and the phylogenies of interacting species that are often taken as partial evidence for coevolution. Here I show that the usefulness of pattern-based studies is not in demonstrating coevolution, but in testing predictions

about how coevolution is expected to influence the evolution of adaptive traits, specialization, or speciation. While the main focus of this chapter is on coevolution in mutualisms, I have attempted to highlight differences and similarities in which mutualisms and antagonisms are expected to evolve, potentially giving rise to differences in the patterns that they produce. In some cases I turn to antagonisms as exemplars because empirically based exemplars of mutualism frequently do not exist for some coevolutionary concepts.

7.2 A short history of coevolution

Although Darwin frequently used terms such as "mutually adapted" and "coadaptations" to describe coevolved interactions, the father of evolution never actually used the term "coevolution." The inception of the term can be traced back to Charles Mode (1958), who developed the first mathematical model of coevolution. This model described gene-for-gene interactions in parasites and their hosts, in which each gene for host resistance is matched by a gene for parasite virulence. The variable outcomes of interactions between flax plants and flax rust fungus were first attributed to this gene-for-gene matching or mismatching of parasite virulence and host resistance by Flor (1942).

Antagonistic interactions were also the focus of a landmark study by Ehrlich and Raven (1964). In a study that popularized the term "coevolution," they examined the dietary patterns of butterfly larvae and found that they were often specialized on unrelated plants with similar chemical defenses. They envisaged that the evolution of a novel insect counterdefense would allow that insect to gain access to a new suite of potential plant hosts with similar defenses, facilitating diversification and speciation. In response, plants would continually evolve new chemicals to counter the evolution of insect resistance, which may also result in further plant radiation, a process called "escape-and-radiate coevolution" (Thompson 1989). From the pollination of the Madagascan star orchid to the two antagonisms described thus far, these contrasting examples demonstrate the variability in the kinds of interactions and mechanisms which can give rise to coevolution.

The 1970s saw a proliferation of mutualism studies, many of which assumed that apparent matches between interacting partners were based on coevolutionary processes. This prompted Janzen (1980) to appeal for more rigorous usage of the term based upon a clear definition, stressing the point that coevolution requires reciprocal evolutionary change. Perhaps because of the difficulty in demonstrating coevolution in accordance with Janzen's strict criteria, many researchers only appeared to consider it as a major evolutionary driving force in intimate and highly specialized relationships, where complete codependence makes it difficult to ignore its probable importance. Central among these relationships were the classic examples of coevolved mutualisms such as the highly specialized brood site mutualisms of yuccas and figs. Because of their simplicity and pairwise nature, obligately specialized mutualisms and highly specialized parasitisms often served as the primary models for studying the coevolutionary process. Many researchers (e.g. Janzen 1981, Howe 1984) subsequently began to perceive coevolution as unlikely or unimportant if interactions were not highly specialized, obligate, and symmetrical.

The critical thinking of the 1980s led to the recognition that most mutualisms (other than some brood site mutualisms, such as those between figs and fig wasps) involved several interacting species, rather than just a single interacting pair. Recent analytical and visualization techniques also confirmed the early observations that interaction webs tend to be highly asymmetrical (see Chapter 11). For example, plants may form guilds in which several species are pollinated or dispersed by a small number of vectors (sometimes only one). However, each vector itself may visit a wide range of plant species (Faegri and van der Pijl 1979). Though this relative complexity complicates our ability to quantify, model, or visualize the reciprocal effects of coevolution on individual populations that interact within multispecific or asymmetric networks, it does not negate the possibility that coevolution is taking place within complex or asymmetrical interactions.

7.2.1 Geographic mosaic of coevolution

After the 1980s, the multispecific and geographically variable nature of many interactions was

cleverly taken advantage of to study coevolution in a geographic context. This idea, developed by John Thompson and his colleagues, is usually referred to as the geographic mosaic of coevolution. Three of Thompson's books document a plethora of studies on the many ways in which geography influences coevolution (Thompson 1994, 2005, 2013). Because

of the inherently heterogeneous nature of the earth's biotic and abiotic surface, all species are faced with a complex mosaic of geographically and temporally divergent selective pressures (Thompson 1997). This geographic context provides us with a beautifully replicated framework with which biologists have been able to study coevolution (see Thompson
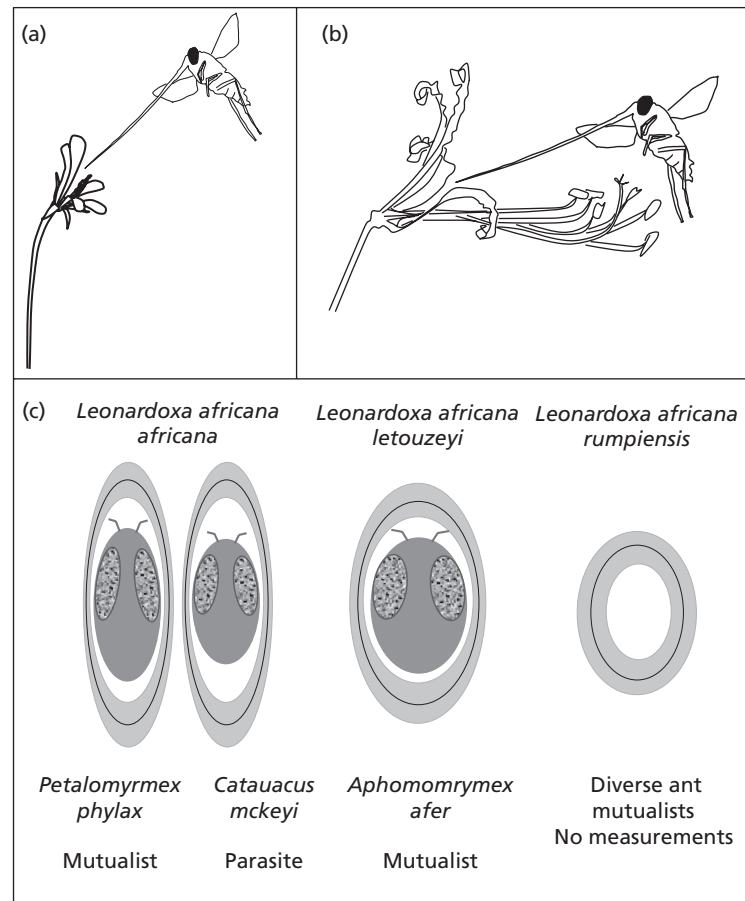

Figure 7.1 Few studies have attempted to demonstrate whether traits facilitating specialization have evolved through processes of filtration versus exploitation. A) In order to access the nectar at the bottom of the floral tube, the long proboscid fly will be required to insert its proboscis deep into the tube. A tube such as this could have evolved to filter out less effective pollinators with shorter proboscides or alternatively it could have evolved to better exploit the morphological characteristics of certain pollinators. The long mouthparts of the pollinators are most likely to have evolved to exploit certain long-tubed flowers more efficiently, suggesting that the matching of traits could have been the result of coevolution. B) The elongated floral parts of *Nerine humilis* could only have evolved to exploit the unusual body parts of its primary pollinator (*Proseoca ganglbaueri*) more efficiently by placing pollen on the fly's abdomen. The filtering out of less efficient pollinators can be ruled out because the nectaries of the flower can be reached by long- and short-tongued pollinators (there is no corolla tube). The flies show no specific adaptations to forage from these flowers, suggesting a unilateral rather than a coevolutionary relationship. C) The differing prostoma shapes (means in black and standard errors in grey) of three subspecies of *Leonardoxa africana* plants effectively filter out ants with larger head sizes, allowing only a certain subset to colonize the plants. One subspecies (*Leonardoxa africana rumpiensis*) is colonized by a diversity of ant species and consequently exhibits a more variable size of entrance hole. In contrast, if the head shapes of the ants have evolved in response to the plants, they could only have evolved to exploit certain species better. Adapted and printed with permission from Brouat et al. (2001).

1999). The geographic mosaic of coevolution recognizes that organisms are unlikely to interact with exactly the same communities throughout their range. Thus, in one population reciprocal selection between two species may be strong, while in another, it may be weaker because it occurs in a different community context. In other populations the same two species may not even co-occur, and organisms may interact with entirely different suites of partners. Under these biotically variable conditions we expect that the magnitude and symmetry of reciprocal selection should be geographically and temporally variable, resulting in a broad mosaic of outcomes that include coevolution in some populations but one-sided evolution and the lack of evolution in others, or mutualism in some populations but antagonism in others (Thompson 1997).

An example of a relationship with geographically variable outcomes due to changes of interacting community members are woodland stars (*Lithophragma parviflorum*) and their pollinators. Woodland stars are very effectively pollinated by the moth *Greya pollitella* (Thompson and Pellmyr 1992). However, this is a brood site mutualism: the moths also lay eggs in some of the plant's ovules, and their larvae consume a small proportion of the developing seeds upon hatching (Thompson and Pellmyr 1992, Pellmyr and Thompson 1996). In some populations, effective copollinators such as bombyliid flies are abundant. They pollinate but do not lay eggs in the flowers (Pellmyr and Thompson 1996). Although both bombyliid flies and *Greya* moths provide benefits to the plants, a plant that is well visited by bombyliids alone should produce a greater seed yield than one equally pollinated by *Greya* moths alone, because the bombyliids do not parasitize any of the seeds. Thompson and Cunningham (2002) showed that the relationship between *Greya* and *Lithophragma* is geographically and temporally variable: when bombyliids were abundant, the relationship between *Lithofragma* and *Greya* is antagonistic, but when bombyliids were scarce, *Greya* and *Lithofragma* are mutualistic.

These data (and others, e.g. Schemske and Horwitz 1984) suggest that when a single species interacts with several others, the participants within those multispecific assemblages are seldom equal in their contributions. In particular, certain species are likely to offer better or more consistent rewards than others. While inefficient partners may still provide a service when considered in isolation, they may have a net antagonistic effect if they inefficiently use resources which would otherwise have attracted more efficient partners. As a result, specialization may evolve in mutualisms, either to better exploit a particular partner (e.g. Stebbins 1970) or to prevent interactions with less efficient partners (e.g. Johnson et al. 2006, Brouat et al. 2001). Figure 7.1 illustrates the subtle differences between these two concepts using an ant–plant mutualism and a pollination mutualism. The ant–plant relationship (Figure 7.1), and the pollination of *Greya* both demonstrate that the geographic mosaic of coevolution can potentially generate a wide array of interaction outcomes. This makes it easy to envisage how coevolution may translate into one of the most important diversifying forces on the planet.

7.3 Demonstrating coevolution

Modeling approaches have demonstrated that coevolution can, at least in theory, affect the traits of organisms interacting in both reciprocally specialized contexts (e.g. Gandon et al. 1996) and highly asymmetrically specialized contexts (e.g. Nuismer at al 2013, Guimarães et al. 2011). Furthermore, coevolution has been invoked to explain the outcomes of interactions that include both mutualisms and antagonistic relationships (Nuismer et al. 1999, Yoder and Nuismer 2010). However, because the kinds of patterns associated with coevolutionary processes can often also be produced by non-coevolutionary processes (Nuismer et al. 2010; see also Section 6.4), demonstrating patterns consistent with the coevolutionary process typically only constitutes partial evidence for its existence. Incontrovertible empirical evidence for the importance or existence of coevolution has been difficult to obtain. To demonstrate coevolution between two organisms, a good starting point would be to identify putatively coevolved traits which are variable, and then show that there is a predictable adaptive fit between the traits of the interacting mutualists. While this sounds like a simple step, it may frequently be the most difficult hurdle. For example, it is hard to pinpoint and quantify coevolving traits in some of

the most tightly associated mutualisms for which many would not hesitate to implicate a history of coevolution (e.g. lichens, coral/algae mutualisms). The next step would be showing that there is selection operating on the identified traits of each partner, and that in each case, the selective agent acting on the trait is the putatively coevolving partner. Finally, one needs to demonstrate that traits in each species have changed as a result of the interaction. While coevolutionary studies typically concentrate

on phenotypic change, it is also possible to study the reciprocal change in the genotypes of interacting organisms. Similar to phenotypic studies, most studies linking adaptation and genetics in mutualisms have typically concentrated on a single mutualist partner (see Chapter 5). However, Noah Whiteman (Box 7.1) describes an exciting future for coevolutionary genomics, in which the changes in the genotypes of both interacting organisms can be studied.

Box 7.1 Co-evolutionary genomics

Noah Whiteman

In the same way that one can quantify reciprocal phenotypic change to demonstrate coevolution, it may also be possible to demonstrate coevolution through the reciprocal changes in allele frequencies driven by natural selection. Advances in the field of genomics, which encompasses classical genetics, quantitative genetics, and population and comparative genomics, are allowing for the discovery of interacting genes in pairs or communities of interacting species that mediate coevolutionary outcomes. Furthermore, the evolution of traits can be facilitated or constrained by their underlying genetic architecture, which includes how many loci and alleles code for a trait, which other traits those loci code for, and how those loci interact with other loci. It is clear that the outcomes of coevolutionary interactions (e.g. winners and losers of coevolutionary races) could depend on the underlying genetic architecture of those traits. Genomics approaches are likely to bear the most fruit when considering relatively specific interactions between hosts and symbionts that infect their hosts, whether the outcome is mutualistic or parasitic. This is principally because of the difficulties associated with identifying clear agents of selection when coevolution is diffuse. The field of coevolutionary genomics is most likely to be advanced by focusing on experimentally tractable, genome-enabled species with well-matched generation times.

Potential signatures of coevolutionary interactions have frequently been studied by observing change or patterns in the phenotypic traits of interacting organisms, but the complementary approach of studying coevolving genes is still in its infancy. However, most genomic studies have been on antagonistic relationships and have only identified the putatively coevolving genes in one of the interacting species.

For example, Dobler et al. (2012) demonstrated that the same agent of natural selection (plant toxins that block the sodium pump, an essential transmembrane carrier) appears to have driven the fixation of identical amino acid substitutions in the sodium pump enzyme (Na,K-ATPase) of distantly related but specialized insects that attack milkweeds. It is less clear whether the insects have in turn caused the host plants to evolve new resistance strategies in response to the evolution of these traits, making coevolution a possible but not definite driver of the genetic changes in the insects. This pattern of convergence at the molecular level is very similar to that observed in sodium channels of snakes that have evolved resistance to tetrodotoxins produced by prey species (Feldman et al. 2012). Although the evidence for coevolution is, perhaps, stronger in the latter case than in the milkweed–insect example, the genomic targets of snake-driven selection have not been characterized in newts that serve as prey for snakes (Brodie et al. 2002, 2005). Nonetheless, progress in identifying the actual genes underlying variation in traits that underlie species interactions has been slow. Why is this the case? Interactions between host and symbiont genotypes give rise to new phenotypic outcomes that are emergent properties of interacting alleles (Wade 2007). Thus, the genes that underlie coevolving traits require the identification of at least two loci (one to several loci per species). This is a difficult task even in one species. However, a good example of how whole-genome sequences of two interacting species can reveal potential patterns of coevolution (that can then be tested by further study) is the metabolic complementarity between aphids and their *Buchnera* spp. endosymbionts (International Aphid Genomics 2010). Furthermore, analogies with epistatic interactions (when expression of one gene depends on expression of another) between genes within the genome of one species can be

Box 7.1   *Continued*

drawn between genes that interact in two different organisms. Wade (2007) has proposed that cotransmission of host and symbiont alleles across generations can eventually lead to mutualistic interactions between species because the fate of one allele in the host depends on the fate of the other in the symbiont, and potentially vice versa. A similar argument has been proposed for the attenuation of virulence in parasites with vertical transmission (Clayton and Tompkins 1994). The potential for such interactions can be measured in many systems in which genetic markers are available by estimating a cotransmission parameter theta (Wade 2007).

A different conceptual approach comes from the field of quantitative genetics. Interactions between host and symbiont genomes are often ignored in typical quantitative genetic models. Consequently, important genetic variation underlying infection outcomes is often overlooked. A different gap exists in coevolutionary biology. Studies aiming to identify the genetic bases of host–symbiont interactions have focused on long-standing gene-for-gene models in which a few loci of major effect interact (such as the examples involving predator–prey and plant–herbivore interactions) in two interacting species to mediate infection or

associational outcome (Flor 1971). However, the heritability of most organismal traits is due to quantitative genetic variation, in which many genes of small effect explain phenotypic variation (Yang et al. 2010; Chapter 5).

Although quantitative genetics has been applied to understand antagonistic coevolved systems, the actual genetic polymorphisms underlying infection or associational outcomes have seldom been identified (Lambrechts et al. 2005, 2006, 2009). Progress in this field requires application of new quantitative models to host–symbiont systems in which the genomic architecture of the association can be dissected in each species. An elegant quantitative genetic model has been proposed by Lambrechts (2010) that could facilitate the identification of interacting genetic polymorphisms in the genomes of coevolving host and symbiont species. In this framework (Box 7.1, Figure 1), infection outcomes are determined by three main terms: (1) additive genetic variation in the host (G_H), (2) additive genetic variation in the parasite (G_P), and (3) variation from interactions between host and parasite genotypes (G_{HP}). However, partitioning of variance in this manner still does not yield the expected genetic polymorphisms. This approach still requires more in-depth

Lambrechts et al. (2006) extends the classic framework in which phenotypes are the summed effects of genes (G), the environment (E), and the interaction between genes and environment (G × E). For simplicity, the G × E and G × G × E interactions are not considered. This is given by :

$$y = \mu + g + \varepsilon$$

where y is the phenotype of an individual, μ is the average population value, g is the genotypic value and ε is the environmental value. If an interaction occurs between symbiont and host, the model is extended to:

$$y = \mu + g_H + g_P + g_{HP} + \varepsilon$$

where g_H is the host genotypic value, g_P is the symbiont's genotypic value and g_{HP} is the genotypic value of the G x G interaction between host and symbiont. Additive and epistatic interactions allow simultaneous consideration of additive and epistatic effects of both host and symbiont loci:

$$y = \mu + \sum \alpha_{HP} + \sum \beta_{HP} + \varepsilon$$

where $\sum \alpha_{HP} = \sum \alpha_H + \sum \alpha_P$ is the sum of the additive effects of loci in host and symbiont, and $\sum \beta_{HP} = \sum \beta_H + \sum \beta_P + g_{HP}$ is the sum of the interaction effects between loci within host and symbiont genomes and interaction effects between host and symbiont genomes.

All eqns. are keyed

Box 7.1 Figure 1 A quantitative, coevolutionary genetics framework for host–parasite interactions.

Box 7.1 *Continued*

studies through genome-wide association (GWA) studies, classic mapping strategies, and functional genetic studies.

Pea aphids and their endosymbiotic *Buchnera* bacteria (Vogel and Moran 2011), as well as *Medicago* spp. plants and their rhizosphere bacteria (Gorton et al. 2012), are both excellent genome-enabled systems in which to explore the genetic bases of mutualisms. However, coevolutionary studies in these systems are challenging to realistically carry out, principally because of the mismatch in generation time between host and symbiont. Conversely, from a practical perspective, studies in which host and symbiont are more evenly matched in terms of life cycle are likely to hold the most promise in the context of identifying gene × gene interactions that mediate coevolutionary outcomes. The study of interactions between microbial species is potentially one way to cope with this issue. For example, communities of microbes living in extreme environments, in which mutualistic interactions may be rampant, are already the focus of intensive study (Stams and Plugge 2009).

In conclusion, the future is bright for those interested in closing the phenotype-genotype-fitness loop for coevolved species and subtending traits that are the targets and agents of reciprocal natural selection. This enthusiasm is due to the rapid progress in the development of new genomics tools, new theory, particularly in quantitative genetics, and the availability of new experimental model systems for the study of coevolution that involve complex eukaryotic species rather than microbes, which have received most attention by researchers (Gloss et al. 2013). However, because the field relies on historical associations between organisms, illuminating the processes that led to current patterns is likely to remain extremely challenging. Clever approaches, such as leveraging of quiescent life stages that can be resurrected (e.g., *Daphnia* and its microparasites), could lead to breakthroughs because the genomes of previous generations can be directly interrogated and could help give insight into current genomic patterns (Decaestecker et al. 2007). Similarly evolve and resequence approaches (Turner et al. 2011) that involve host–symbiont or predator–prey interactions are likely to be extremely illuminating because loci detected as being subject to selection can be screened in natural populations. Thus, the pairing of experimental genomics and landscape genomics could be a powerful approach to validate results generated in the laboratory. The challenge, however, is in determining the nature of criteria required to prove that coevolution has occurred, and the availability of genomic data have not made this task any easier.

References

Brodie, E.D. 3rd., Feldman, C.R., Hanifin, C.T., Motychak, J.E., Mulcahy, D.G, Williams, B.L., and Brodie, E.D. Jr. (2005). Parallel arms races between garter snakes and newts involving tetrodotoxin as the phenotypic interface of coevolution. *Journal of Chemical Ecology*, **31**, 343–356.

Brodie, E.D. Jr., Ridenhour, B.J., and Brodie, E.D. 3rd. (2002). The evolutionary response of predators to dangerous prey: hotspots and coldspots in the geographic mosaic of coevolution between garter snakes and newts. *Evolution*, **56**, 2067–2082.

Clayton, D.H., and Tompkins, D.M. (1994). Ectoparasite virulence is linked to mode of transmission. *Proceedings of the Royal Society of London B*, **256**, 211–217.

Decaestecker, E., Gaba, S., Raeymaekers, J.A., Stoks, R., Van Kerckhoven, L., Ebert, D., and De Meester, L. (2007). Host-parasite "Red Queen" dynamics archived in pond sediment. *Nature*, **450**, 870–873.

Dobler, S., Dalla, S., Wagschal, V., and Agrawal, A.A. (2012). Community-wide convergent evolution in insect adaptation to toxic cardenolides by substitutions in the Na,K-ATPase. *Proceedings of the National Academy of Sciences USA*, **109**, 13040–13045.

Feldman, C.R., Brodie, E.D. Jr., Brodie, E.D. 3rd., and Pfrender, M.E. (2012). Constraint shapes convergence in tetrodotoxin-resistant sodium channels of snakes. *Proceedings of the National Academy of Sciences USA*, **109**, 4556–4561.

Flor, H.H. (1971). Current status of the gene-for-gene concept. *Annual Review of Phytopathology*, **9**, 275–296.

Gloss, A.D., Nelson Dittrich, A.C., Goldman-Huertas, B., and Whiteman, N.K. (2013). Maintenance of genetic diversity through plant–herbivore interactions. *Current Opinion in Plant Biology*, **16**, 443–450.

Gorton, A.J., Heath, K.D., Pilet-Nayel, M.L., Baranger, A., and Stinchcombe, J.R. (2012). Mapping the genetic basis of symbiotic variation in legume–rhizobium interactions in *Medicago truncatula*. *G3: Genes, Genomes, Genetics*, **2**, 1291–1303.

International Aphid Genomics, C. (2010). Genome sequence of the pea aphid *Acyrthosiphon pisum*. *PLoS Biology*, **8**, e1000313.

Lambrechts, L. 2010. Dissecting the genetic architecture of host-pathogen specificity. *PLoS Pathogens*, **6**, e1001019.

Lambrechts, L., Chevillon, C., Albright, R.G., Thaisomboonsuk, B., Richardson, J.H., Jarman, R.G., and Scott, T.W. (2009). Genetic specificity and potential for local adaptation between dengue viruses and mosquito vectors. *BMC Evolutionary Biology*, **9**, 160.

Lambrechts, L., Fellous, S., and Koella, J.C. (2006). Coevolutionary interactions between host and parasite genotypes. *Trends in Parasitology*, **22**, 12–16.

Lambrechts, L., Halbert, J., Durand, P., Gouagna, L.C., and Koella, J.C. (2005). Host genotype by parasite genotype interactions

Box 7.1 *Continued*

underlying the resistance of anopheline mosquitoes to *Plasmo-dium falciparum. Malaria Journal*, **4**, 3.

Stams, A.J.M., and Plugge, C.M. (2009). Electron transfer in syn-trophic communities of anaerobic bacteria and archaea. *Nature Reviews Microbiology*, **7**, 568–577.

Turner, T.L., Stewart, A.D., Fields, A.T., Rice, W.R., and Tarone, A.M. (2011). Population-based resequencing of experimen-tally evolved populations reveals the genetic basis of body size variation in *Drosophila melanogaster. PLoS Genetics*, **7**, e1001336.

Vogel, K.J., and Moran, N.A. (2011). Sources of variation in dietary requirements in an obligate nutritional symbiosis. *Proceedings of the Royal Society of London B*, **278**, 115–121.

Wade, M.J. (2007). The co-evolutionary genetics of ecological com-munities. *Nature Reviews Genetics*, **8**, 185–195.

Yang, J., Benyamin, B., McEvoy, B.P., Gordon, S., Henders, A.K., Ny-holt, D.R., Madden, P.A., Heath, A.C., Martin, N.C., Montgomery, G.W., Goddard, M.E., and Visscher P.M. (2010). Common SNPs explain a large proportion of the heritability for human height. *Nature Genetics*, **42**, 565–569.

Some phenotypic studies of coevolution in mutu-alisms satisfy a few of these criteria, but none of them do a good job of satisfying all of them. Based on Darwin's ideas on coevolving plant and pollin-ator traits, the quantification of pollinator foraging apparatus (e.g. pollinator proboscis length) and the tubular structures of flowers (e.g. corolla tubes) have been well studied in many systems and sev-eral studies suggest instances where the traits of pollinators and plants appear to be predictably matched (e.g. Steiner and Whitehead 1991, Ander-son and Johnson 2008, Figures 7.2 and 7.3). Simi-larly, in some African fig pollination mutualisms, large figs tend to be associated with female fig wasp pollinators with elongated heads, while smaller figs are pollinated by wasps with shorter, more square-shaped heads. The elongated heads are thought to be an adaptation for pushing through the longer ostioles of the large figs. Because male wasps do not enter fig ostioles, the head size–fig size relation-ship is not strong for the male wasps. However, it is strong for unrelated, non-pollinating wasp taxa which enter the figs as parasites (van Noort and Compton 1996). Ovipositor lengths of fig wasps are also frequently closely matched to the style lengths of the figs (Weiblen 2004).

Several studies have also demonstrated selection on traits that are putatively coevolving; however, few studies have quantified selection acting on the traits of both organisms. For example, many stud-ies have documented positive directional selection on floral tube or spur lengths, suggesting partial evidence in support of Darwin's coevolutionary race (e.g. Nilsson 1988, Alexandersson and Johnson 2002, Muchhala and Thomson 2009, Anderson et al. 2010a, Ellis and Johnson 2010). However, none of these studies investigated selection on the proboscis lengths of their pollinators. Because it is often hard to estimate reproductive success (i.e. to measure fitness) from the fleeting encounters of free-living partners such as pollinators or seed dispersers, quantifying selection gradients on them is a major hurdle. Furthermore, demonstrating correlations be-tween fitness and trait magnitudes usually does not show explicitly that the agent of selection is the puta-tively coevolving partner. Instead, selection studies often show only that a measure of fitness is associ-ated with the magnitude of a particular trait (e.g. Anderson et al. 2010a). Using a pollination mutual-ism, Pauw et al. (2009) elegantly circumnavigated some of these problems by measuring the effects of floral tube length on the efficiency of pollen receipt after visitation by pollinators with a known probos-cis length. Simultaneously, the authors were able to measure the amount of nectar that pollinators with known proboscis lengths were able to consume from flowers of known tube length. This enabled them to show that tube lengths and proboscis lengths were both the agents as well as the targets of reciprocal, directional selection. Left open to debate is whether pollen receipt and nectar consumption are accurate measures of fitness. In fact, the difficulty of quan-tifying total fitness measurements is a weakness of many of the aforementioned selection studies.

Another problem with selection studies is that even if reciprocal selection is demonstrated, it is still

difficult to ascertain how important coevolution was as a force driving the evolution of a particular trait. For example, the hummingbird-pollinated plant *Nicotiana glauca* has long-tubed flowers, which are probably adapted to hummingbird beak dimensions (Nattero and Cocucci 2007). Although native to Argentina, the plant is a successful invader in many parts of the world, including southern Africa, where unrelated but functionally similar sunbirds probe the flowers with their long beaks (Geerts and Pauw 2009). The long, tubular corolla is a useful pre-adaptation to sunbird pollination, and may well be under present selection by the sunbirds. However, selection by sunbirds clearly did not play a role in the evolution of long tubes in *Nicotiana*. Sunbirds may, however, select for slight modifications in the tube dimensions. Consequently, the interpretation of selection studies needs to be carefully considered within the context of the interaction. This example also demonstrates the importance of showing that traits have changed in response to their interacting partners.

Among-population trait divergence and phylogenetically controlled analyses of trait divergence have both been used to demonstrate evolutionary change (e.g. Weiblen 2004, Chapter 3). However, it is still difficult with this approach to ascertain whether one or both species have undergone evolutionary change in response to one another. Some studies have attempted to combine trait variation approaches with selection or local adaptation studies in order to demonstrate which traits are likely to have changed in response to one another (e.g. Anderson and Johnson 2008, Pauw et al. 2009). A series of powerful studies document sexual dimorphism in the bill shapes of purple-throated carib hummingbirds. Two species of *Heliconia* are important nectar sources for the hummingbirds, and in sympatry they usually have different tube shapes which are advertised with different floral colours (Temeles et al. 2000). Each hummingbird sex preferentially feeds from *Heliconia* flowers that fit their bill morphology, and it appears that the fit between bill and flower morphology also affects the foraging efficiency of the birds (Temeles et al. 2000, Temeles and Kress 2003). This has been interpreted as evidence for evolutionary change by the birds in response to the different feeding niches presented by

Heliconia (Temeles et al. 2000). Each species of *Heliconia* is, however, polymorphic for floral colour and corolla morphology, and the relative prevalence of each morph is dependent on variations in the floral community context (Temeles and Kress 2003). Floral polymorphisms in the two *Heliconia* have been interpreted as evolutionary responses to the two pollinator niches. Consequently, coevolution in this system is supported by evidence for evolutionary responses of hummingbirds to *Heliconia* flowers as well as evolutionary responses of *Heliconia* flowers to humingbirds (Temeles and Kress 2003).

Another powerful tool in studying morphological change is the documentation of phenotypic responses of interacting organisms over short time scales. Galetti et al. (2013) demonstrated that a hundred years after the loss of large-gaped seed dispersers, palm populations had evolved smaller seeds compared to populations which had intact seed dispersing fauna (a case of unilateral evolutionary change). Although the nature of this study made it impossible to look at reciprocal changes in bird morphology, putatively reciprocal change has been demonstrated in interactions where both interacting partners have fast generation times. Mostly such studies have been on host–parasite systems involving annual plants or rapidly producing invertebrates and their pathogens (e.g. Thrall et al. 2012, Koskella and Lively 2007). An advantage of these fast-generation systems is that it is often easy to estimate fitness in both partners through measurements of mortality and virulence. Resurrection ecology, where it is possible to "travel back in time" by resurrecting organisms trapped in old strata, has also produced some good evidence for reciprocal evolutionary change (Decaestecker et al. 2007). Here it is possible to trace temporal changes of resistance and virulence phenotypes, as well as to infect ancient hosts with more recent parasites and vice versa (e.g. Decaestecker et al. 2007). Presently, real-time coevolution and resurrection studies typically focus on antagonistic relationships. However, Weeks et al. (2007) examined *Wolbachia* infections of *Drosophila* in which *Wolbachia* appear to have rapidly evolved a mutualistic relationship from a previously parasitic one (see Box 2.1 and Chapter 3). Initially *Drosophila* fecundity was reduced by *Wolbachia* infections, but after twenty years of evolution, the fecundity of infected *Drosophila* was higher than for

uninfected *Drosophila*. So far, evolutionary changes only appear to have been made on the part of the *Wolbachia* (i.e. unilateral evolution rather than coevolution has taken place). However, this does not preclude the possibility that future evolutionary changes in the *Drosophila* may facilitate infection by the mutualistic *Wolbachia*.

Although unequivocal evidence for coevolution is rare, partial evidence consistent with coevolution in both mutualisms and antagonisms is so widespread that, taken together, it may be hard to imagine a world in which coevolution had no role to play (Thompson 1994, 2005). In situations where the phenotypic variability of organisms affects each other's fitness on a routine basis, coevolution is most likely at work, either making fine or major adjustments to the genotypes and phenotypes of those interacting organisms. In many cases, perhaps, the questions we need to ask are not whether coevolution has occurred, but whether the products of coevolution are consequential, and whether coevolution can lead to quantifiable trait change. To do this we need to have a clear idea of what patterns in nature could conceivably be generated by coevolution.

7.4 Coevolutionary patterns

Coevolutionary patterns include the ways in which matches or mismatches between putatively coevolving traits occur in time and space, as well as the phylogenetic patterns of coevolving clades. Some of the earliest putative examples of coevolution appear to be based on the close phenotypic matching of traits between interacting organisms. As we have seen, Darwin (1862) himself invoked the idea of coevolution as the potential mechanism behind the matching lengths of hawkmoth tongues and orchid spurs. However, while this kind of matching may well be the result of coevolution, trait-matching cannot prove coevolution; similarly, a mismatch cannot disprove coevolution (Janzen 1980, Nuismer et al. 2010). In fact, broad-scale patterns of matching traits may be expected whenever it is advantageous for at least one partner (not necessarily both) to adapt to the traits of the other. This is exemplified in Figure 7.2, where similar patterns of trait matching are demonstrated in a putatively coevolved

species pair and in a pair of species where the traits are unlikely to have evolved through coevolution. Trait matching can consequently be expected in mutualisms, antagonisms, and commensalisms (Nuismer et al. 2010). However, the type of relationship or the kind of coevolutionary mechanism may affect the degree or likelihood of matching (Nuismer et al. 2010). In this section I will highlight some of the patterns frequently attributed to coevolution, and explain some of the coevolutionary and other mechanisms that may produce them. I will also draw attention to the expected similarities and differences between patterns produced by mutualistic versus antagonistic coevolution.

7.4.1 Matching of traits

7.4.1.1 Coevolutionary complimentarity

Despite potential conflicts of interest between mutualists (see Chapter 6), traits may frequently coevolve to facilitate mutual access to rewards and services. Consequently, there is often selection for a certain degree of trait complimentarity between partners. Mutual recognition systems between nitrogen-fixing rhizobial bacteria and the plants they colonize (Miklasheviches et al. 2001) provide an example of trait complimentarity in mutualism. The plants involved in this interaction (described in more detail in Box 5.2) control the pathway for the production of a key amino acid used by the bacteria, while bacteria control the pathway for the production of a key amino acid used by the plants (Lodwig et al. 2003). It is thought that the mutualism is stabilized because neither the plant nor the bacteria can dominate the relationship without having an inverse effect on fitness. Another example of trait complimentarity can be found in the mutualistic interaction between the tree *Leonardoxa africana* and certain ants that protect it from herbivory (Figure 7.1). The tree produces modified thorns in which the ants live (domatia), but the ants need to chew through a weak, unlignified spot called a prostomium in order to gain entry (Brouat et al. 2001). Different populations of *L. africana* are associated with different species of ants with different head shapes. The shapes and sizes of the associated ants are closely matched to the shapes and sizes of the prostomia. It is evident that the prostomia act

as a filter allowing a small subset of ants to interact with the plant (Brouat et al. 2001). Phylogenetic analyses support the idea that the head shapes of some ant species are highly derived, suggesting coevolutionary origins. While trait matching can also be observed in antagonistic relationships, its root causes are usually different.

7.4.1.2 Coevolutionary races

Unlike mutualisms, antagonisms involve individuals of one species that usually experience a net loss in fitness as a result of the interaction, while individuals of the other species usually gain fitness. Consequently one may expect the "losing" species to evolve mechanisms enabling it to escape the interaction through the mismatching of traits, while the "gaining" species should evolve mechanisms to match the traits of the "losing" species in order to continue benefiting from the interaction (Thompson 2013). This dynamic engenders an "escape-and-chase" scenario. The oscillatory cycles of coevolving polymorphisms are a good example of escape-and-chase coevolution. Gene-for-gene coevolution takes the format of oscillating polymorphisms for resistance genes in the host, and virulence genes in the parasite. Fluctuations in resistance and virulence gene frequencies are underpinned by negative frequency dependent selection, in which rare parasite phenotypes are not easily recognized by hosts, making them more infectious than more common phenotypes. However, as they become more common, selection on the host for defense against that phenotype increases. If each infection phenotype is coded for by a specific genotype, and each resistance phenotype is coded for by a specific resistance genotype, the expectation is that different genotypes for infection and resistance will cycle through time, sometimes with close infection-resistance phenotype matching (e.g. Thrall and Burdon 2003), but occasionally with host and parasite phenotypes being completely mismatched and out of phase (e.g. Thrall et al. 2012). In these types of relationships, the frequency of mismatched versus matched traits could be dictated by how fast the parasite evolves compared to the host. Frequent mismatches favoring the host may be expected with a relatively quickly evolving host, but close matching of resistance to virulence

phenotypes (favoring the parasite) would be expected if the parasite evolved relatively quickly compared to the host.

While the oscillatory races of gene-for-gene coevolution have never been documented for mutualisms, the antagonistic aspects of mutualisms may give rise to escalatory races between coevolved traits. The most famous example of this is Darwin's race (described in Section 7.1), where a positive feedback system is generated by increases in floral tube length selecting for increases in pollinator tongue length which in turn selects for increases in tube length. This leads to a race in which traits become larger or more extreme. Clearly, such a race of exaggeration cannot continue indefinitely: the race is halted when an opposing selection force equals and stabilizes the selection force for further exaggeration in the traits of either partner. For example, trait elongation on hawkmoth proboscides may stop if it becomes too difficult for the hawkmoth to maneuver a proboscis in excess of a certain length. We would then expect the plants to evolve spurs which are marginally longer than the moth tongues, forcing the moths to make contact with the reproductive parts of the flowers whenever they visit. In putatively coevolving mutualisms, traits that are expected to coevolve under this kind of model have frequently been found to vary geographically. For example, three-fold variation in the proboscis length of a long-proboscid fly species was demonstrated across its range (Anderson and Johnson 2008). Similar variation has been observed for other species of long-proboscid fly (Pauw et al. 2009, Newman et al. 2014) as well as for the forelegs of *Rediviva* bees, which use their legs to mop up oil from the deep, twin spurs of oil-producing *Diascias* (Steiner and Whitehead 1991). In each of these systems the lengths of the floral parts correlate with the lengths of the pollinator parts (see Figure 7.2 for correlations between pollinator foraging traits and floral tube length). Similar correlations have been found in antagonistic relationships where escalatory coevolution has been invoked to explain the covariation in toxicity and resistance of newts and their garter snake predators respectively (e.g. Hanifin et al. 2008), as well as the evolution of extremely extended mouthparts in weevils and the correspondingly thick pericarps of their host plants

(e.g. Toju and Sota 2006). While correlated patterns of trait exaggeration may be a frequent outcome of coevolution, they alone do not prove that coevolution is or has been occurring. This is because correlated traits can also be an outcome of unilateral evolution where one species adapts to another but not vice versa (Figure 7.2). Trait correlations could also conceivably occur if abiotic selective pressures select on the traits of both interacting species (see Nuismer et al. 2010 for further discussion on this topic). Patterns of trait matching may, however, be used to test whether matching is stronger in mutualisms versus parasitisms or commensalisms (e.g. Anderson et al. 2010b; see Section 7.4.2).

7.4.2 Mismatching of traits

7.4.2.1 Asymmetries in escalatory races

Even though escalatory races may produce correlated traits, correlations do not necessarily mean that the traits are well matched. Two kinds of mismatches can be distinguished in trait matching studies. The first is that regressions between traits are never perfect. There are always some populations of interacting individuals that appear to match better than others (Anderson et al. 2010b). Variability in the degree of mismatch between populations of interacting species may be the result of variability in the way in which populations respond to each
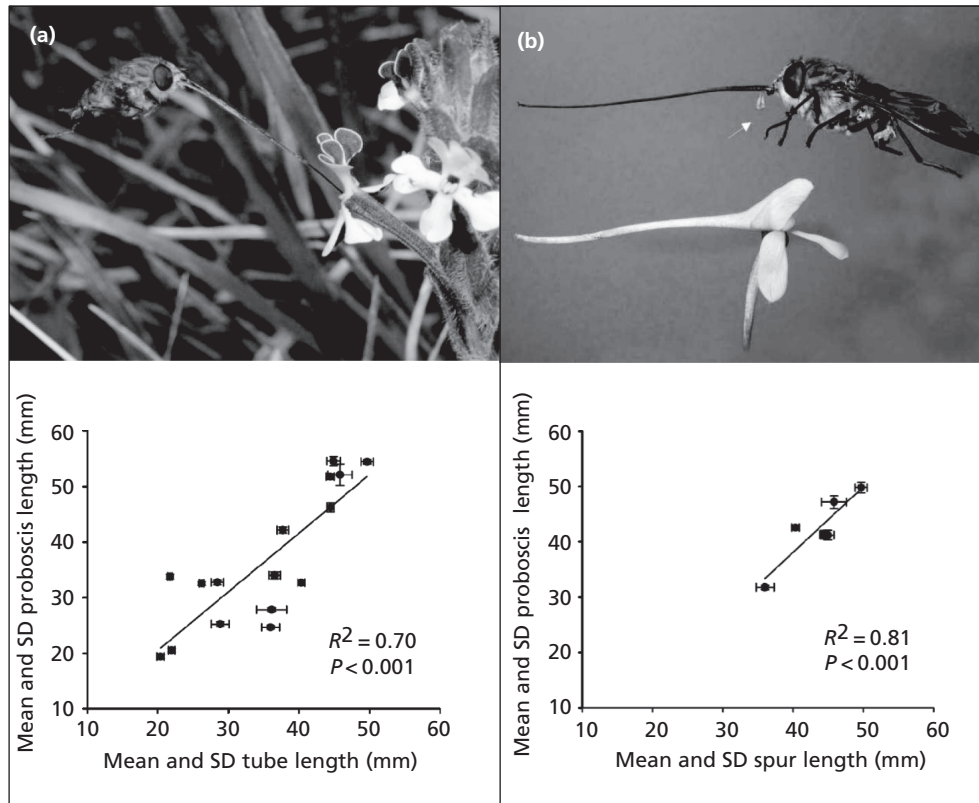

Figure 7.2 Similar patterns of trait matching between mutualistic, coevolved species and antagonistic, unilaterally evolved species. A) *Zaluzianskya microsiphon* is the principal nectar source for the pollinator (*Prosoeca ganglbaueri*), whose probosicis length is closely matched to the tube lengths of the plants in each population. B) *P. ganglbaueri* flies are deceitfully attracted to the mimetic orchid *Disa nivea* which has no nectar. Even though the tube length of the floral spur does not select on fly tongue length, the tongue lengths of the flies select on the tube lengths of the plants, generating patterns of trait matching. Long tubes facilitate precise pollen placement at the base of the fly proboscis (see arrow). Reprinted with permission from Anderson and Johnson (2009).

other. One of the pillars of the geographic mosaic of coevolution is that the strength and symmetry of selection between interacting organisms is likely to vary among populations, leading to coevolutionary hotspots and coevolutionary coldspots (Thompson 1994, 1999, 2005).

Regressions of matching traits may also differ in their slopes (Anderson et al. 2010b). Theoretically, we could conceive of a situation where the average trait measurements of interacting populations are perfectly matched, so that on average, neither population has a functional advantage over the other. If these traits were geographically variable but perfectly matched at each site, we could draw a theoretical line through the data points, which we could call the expected line for perfect trait matching. We could then collect real data on trait matching from multiple populations and determine whether the line for perfect trait matching is a good fit of the real data or not. Deviations from the line for perfect trait matching suggest that one species may have the functional "upper hand" in the relationship. The best examples of this are from the parasite and predator–prey literature on coevolution where the values for perfect trait matching were experimentally calculated (e.g. Toju and Sota 2006, Hanifin et al. 2008). Camellia weevils have evolved an extraordinarily long rostrum with chewing mouthparts on the end, used to bore through the thick woody pericarp of Japanese camellias, so that their eggs can be laid on the seeds within. Perfect balance or trait matching is when the rostrum is long enough that half of the beetle's boring attempts within a population of Japanese camellias are successful and the seeds of the camellia are reached (Toju and Sota 2006). Toju and Sota (2006) and Hanifin et al. (2008) identified consistent mismatches in the traits of interacting species, suggesting that winners and losers of coevolutionary races may be predictable.

Ultimately, the dimensions of interacting traits are governed by the net outcome of several selection pressures acting on them, not just the reciprocal selection pressures generated by the interaction. In other words, the traits of interacting organisms are not only shaped by selection driven by each other, but also by constraints selecting against

further trait exaggeration. Often, these constraints are generated from the abiotic environment in which the interaction takes place, and these constraints are likely to be quite different for the interacting organisms. For example, the rostrums of camellia weevils and the pericarps of Japanese camellias exhibit predictable patterns of phenotype mismatching within a strongly correlated pattern of phenotype matching (Toju and Sota 2006). The exaggeration of pericarp thickness is thought to be constrained by resource availability, while the exaggeration of weevil mouthparts is thought to be constrained by allometry (Toju et al. 2011). Consequently, when resources are scarce, pericarp thickness is the first trait to be constrained, and the traits of the weevils exceed those of the plants. At resource-poor sites, the arms race does not proceed very far, and both pericarp thickness and rostrum length are poorly developed. However, when resources are not limiting, the race can continue to much greater lengths. In these populations the increased exaggeration of weevil mouthparts is eventually allometrically constrained, allowing the pericarps to become relatively more exaggerated and the plants to dominate the race (Toju et al. 2011). Increased exaggeration of traits may also be genetically constrained (Box 7.1). For example, Hanifin et al. (2008) found that although newt toxicity and snake resistance were closely correlated, snakes were consistently more resistant to newt toxicity than predicted by a scenario of perfect trait matching. It was hypothesized that the relative success of the snakes was due to the fact that high snake resistance in some populations can be explained by variation of a single nucleotide substitution (Geffeney et al. 2005), making it potentially easier to evolve than newt toxicity.

These advances in the literature on host–parasite coevolution lead to questions about whether similar trait mismatches occur in mutualisms, and whether mismatches in mutualisms tend to be larger or smaller than in antagonisms. We may also start asking questions about which factors contribute most toward trait mismatches. The answers to these questions may enable us to make predictions about the direction of trait mismatches in interacting species and about which species are expected to win coevolutionary races.

While models are an obvious place to start answering such questions, one empirical study examined patterns of trait matching using multiple published data sets on the traits of interacting plants and insects (Anderson et al. 2010b). They found that correlations between traits frequently occurred irrespective of whether the relationships were mutualistic or antagonistic. Although their analyses were crude in comparison to the host–parasite examples cited in this section, they nevertheless found similar mismatches in traits, comparable to those found in the antagonistic systems. Typically, the larger the traits, the more extreme the absolute mismatch; a case in point is that the most extreme mismatch was found for Darwin's famous orchid, with a spur length of approximately 30 cm, and its primary hawkmoth pollinator, with a proboscis length of only about 24 cm. More intriguing was the observation that the direction of trait mismatch evident in Darwin's orchid and hawkmoth appeared to hold for most interacting plant–insect partners across the spectrum of traits examined. In other words, the traits of plants tended to be more exaggerated than the traits of the interacting insects (Figure 7.3). The authors

speculate that asymmetries in the constraints or selective pressures acting on insect versus plant traits were the primary reasons for the patterns of mismatch. However, modeling approaches may prove more useful in dissecting out the real causes of trait mismatches. In Box 7.2, Scott Nuismer models the matching and mismatching of traits. He demonstrates that inherent differences in the selection pressures against further trait exaggeration (i.e. constraints resulting in stabilizing selection) in insects versus plants may be extremely important in generating trait mismatches. Furthermore, he also shows that the relative population sizes of plants and insects can also play an important role in generating trait mismatches.

7.4.3 Trait divergence between sites

The divergence of traits through coevolution is classically associated with competition. For example, two of Darwin's finches competing for food may both diverge in their beak characteristics as they specialize on slightly different resources (e.g. large versus small seeds, *sensu* Grant and Grant 2006). Consequently Darwin's finches in populations
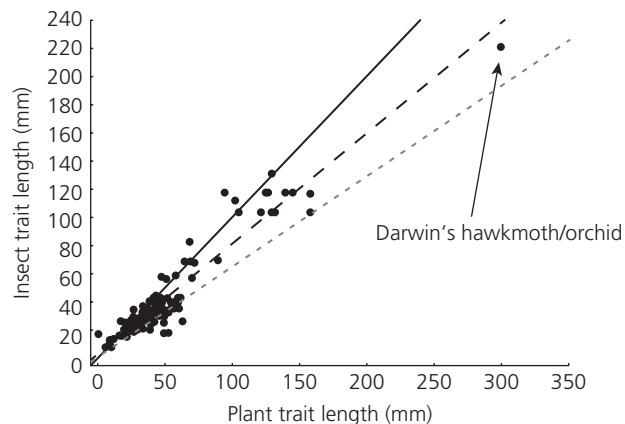

Figure 7.3 Scatterplot showing the relationship (ordinary least squares regression) between putatively complimentary traits for interacting plants and insects. Each data point represents a species mean for an interacting pair of plants and insects where at least one species (usually the plant) is highly specialized on the other. The solid line is the slope of unity or the line where traits are "perfectly matched" and it is hypothesized that neither the plant nor the insect has a functional "upper hand." The black, dashed line is the line of best fit using ordinary least squares regression and suggests that plant traits are typically more exaggerated than insect traits. The gray, dotted line describes a model adjusted for insect phylogeny, effectively magnifying the asymmetries of trait exaggeration. To illustrate the point using an extreme example, Darwin's Madagascan star orchid has a spur length of 300 mm while its principal pollinator has a tongue length of only 230 mm. Reprinted with permission from Anderson et al. (2010b).

Box 7.2 When do trait mismatches coevolve?

Scott L. Nuismer

Recent work exploring the relationship between traits of interacting mutualist species demonstrated that although traits of mutualists tend to be correlated, trait mismatching appears to be common. Specifically, by estimating the slope of the relationship between plant and animal traits for published data sets, Anderson et al. (2010) showed that the traits of plants tended to be more exaggerated than the traits of the interacting insects (Figure 7.3). To explore the possible causes of this pervasive pattern of trait mismatching, I develop a simple model of mutualistic coevolution and use it to predict the slope of the relationship between plant and insect traits.

I model mutualistic coevolution between a pollinating insect species, x, and a plant species which it pollinates, y. I assume the species live within a metapopulation consisting of N individual populations, each of which is inhabited by n_x and n_y insect and plant individuals, respectively. The mutualistic interaction is mediated by a single quantitative trait in each species, with coevolutionary selection favoring phenotype matching with strength S_x and S_y, respectively. In addition, insect and plant species experience stabilizing selection of strength ψ_x and ψ_y favoring trait values θ_x and θ_y respectively. As long as selection is weak and additive genetic variance fixed, this model can be used to study the evolution of trait means (Nuismer et al. 2010, Yoder and Nuismer 2010).

In order to tie the predictions of our model to empirical studies, I calculate the slope of a regression of insect trait on plant trait using reduced major axis (RMA) regression:

$$\beta_{xy} = \frac{\sigma_x}{\sigma_y} \tag{B1}$$

where σ_x and σ_y measure the standard deviations of trait means across the metapopulation for insect and plant, respectively. As long as a dynamic equilibrium between selection and drift has been reached, our model reveals that the slope of this relationship is given by:

$$\beta_{xy} = \frac{\sqrt{G_y n_y \left(S_y + \psi_y\right)^2 + G_x \left(n_x S_x^2 + n_y \left(S_y \psi_x + S_x \psi_y + \psi_x \psi_y\right)\right)}}{\sqrt{G_x n_x \left(S_x + \psi_x\right)^2 + G_y \left(n_y S_y^2 + n_x \left(S_y \psi_x + S_x \psi_y + \psi_x \psi_y\right)\right)}} \tag{B2}$$

Inspection of (B2) reveals several important insights. First, a slope of 1 is the exception rather than the rule. Only for very specific combinations of parameter values should a 1:1 relationship among traits of the coevolving species occur, the

most obvious being when both species are identical with respect to S, G, n, and ψ. Second, in addition to the strength of coevolutionary selection, S, and stabilizing selection ψ, which have been previously hypothesized to shape the slope (Anderson et al. 2010), population sizes n and additive genetic variances G also appear to play an important role.

To gain further insight, I evaluated (B2) numerically. These analyses show that, in general, slopes greater than one are favored when species x has smaller population size than species y, experiences weaker stabilizing and coevolutionary selection than species y, and has less additive genetic variance than species y. Surprisingly, these analyses also reveal that the slope is much more sensitive to asymmetries in local population sizes and strengths of stabilizing selection than it is to asymmetries in the strength of coevolutionary selection (Box 7.2, Figure 1).

Intuitively, the effect of each evolutionary force on the slope can be understood by considering its influence on spatial variability among trait means, σ_i. Specifically, forces reducing spatial variability in species x decrease the slope whereas forces reducing spatial variability in species y increase the slope. Within our simple model, the evolutionary forces that reduce spatial variability include stabilizing selection (which I assume is spatially homogenous), coevolutionary selection, and additive genetic variance (because it facilitates the response to selection). Only random genetic drift increases spatial variability, with this effect greatest for small population sizes.

These results may help to explain why recent studies have observed such frequent mismatches in traits as interpreted by their slopes (Anderson et al. 2010). Specifically, slope deviations in these studies could have been caused by differences in the effective population sizes of plants and insects, differences in the constraints acting on plant and insect traits, or differences in the strength of selection for exaggeration of plant and insect traits. Of these possibilities, the first two seem the most plausible since only very extreme asymmetries in coevolutionary selection could generate the slopes observed in empirical studies whereas modest asymmetries in local population sizes and levels of constraint are consistent with the data (e.g., Box 7.2, Figure 1).

Taken together, the results of this model illustrate that the slope inferred from studies of trait matching is influenced by many evolutionary forces, among which coevolution is but one. The failure of the slope to provide unique and compelling evidence for coevolution raises an obvious question: should we abandon studies of trait matching as a

continued

Box 7.2 *Continued*
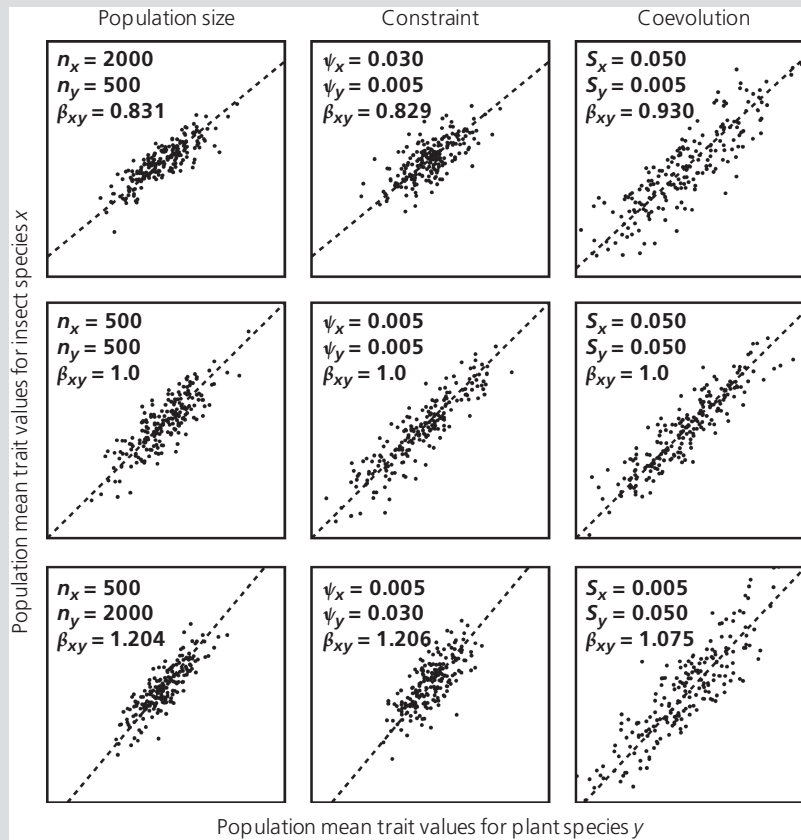
Box 7.2 Figure 1 The slope of the reduced major axis (RMA) regression predicted by the analytical model (B2) shown as a dashed line. The dots represent trait values for individual populations as predicted by a stochastic simulation of a metapopulation consisting of 200 individual populations. Key parameters are shown within the upper left-hand corner of each figure, as is the predicted slope, with the first column demonstrating the effects of relative population size on trait matching slopes, the second column demonstrating the effects of relative constraints upon trait matching slopes, and the third column demonstrating the effects of the relative strengths of coevolutionary selection on trait matching slopes.

failed endeavor? My opinion is that this would be premature. In addition to identifying patterns ripe for mechanistic explanation, studies of trait matching have identified potentially important general trends and serve as a means to study differences in the patterns of mismatching between mutualisms and antagonisms. Identifying the mechanisms generating these patterns will require the development of model-based statistical approaches that can wring the most possible information from existing data sets. If such approaches can generate even coarse estimates for the strength of coevolutionary selection within natural systems

then our understanding of evolving species interactions could be vastly improved.

References

Anderson, B., Terblanche, J.S., and Ellis, A.G. (2010). Predictable patterns of trait mismatches between interacting plants and insects. *BMC Evolutionary Biology*, **10**, 1471–2148.

Nuismer, S.L., Gomulkiewicz, R., and Ridenhour, B.J. (2010). When is correlation coevolution? *American Naturalist*, **175**, 525–537.

Yoder, J.B., and Nuismer, S.L. (2010). When does coevolution promote diversification? *American Naturalist*, **176**, 802–817.

without competitors may have different beak dimensions than in populations of the same species which do have to compete with other finches for food (character displacement). Importantly, intraspecific divergence is the product of a heterogeneous landscape in which populations coexist with mosaics of different competitors.

While coevolving mutualisms are seldom the examples that people turn to when they think about trait divergence, environmental heterogeneity could drive trait divergence of mutualist traits in a similar way. The environments experienced by coevolving mutualists are heterogeneous in terms of the biotic components (communities) in which the mutualisms are embedded, as well as in terms of their abiotic components (Thompson 2005). *Greya politella* visits and pollinates several species of woodland stars across its range. Multiple traits of these flowers (e.g. ovary position, stigma length, floral depth) differ between species (Thompson et al. 2013). The *Greya* moths, now recognized as a species complex consisting of several lineages (Rich et al. 2008), exhibit multiple traits (e.g. ovipositor length, proboscis length, and the length of abdominal segments) that covary with the traits of the plants they are found on. The covariation and divergence of moth and plant reproductive traits appear to maintain the effectiveness of both oviposition and pollination across a variable biotic mosaic (Thompson et al. 2013). Similarly intraspecific covariation of pollinator and plant traits described for escalatory races (Section 7.4.1.2) also appear to vary geographically in response to one another (e.g. Steiner and Whitehead 1991, Anderson and Johnson 2008, Pauw et al. 2009, Newman et al. 2014, Figure 7.2). However, these studies raise the question of why traits are geographically divergent and why the escalatory race does not stop at the same place in all populations, leading to a lack of trait divergence across populations. Once again, studies on antagonisms offer the best empirical evidence for why the escalatory traits vary geographically. For Japanese camellias, it appears as if variability in resource availability plays an important role in halting the escalatory race between the weevils and camellias at different endpoints (Toju et al. 2011). We can borrow from these ideas and hypothesize that different

abiotic conditions could stop escalatory races at different end points for pollination mutualisms too. Perhaps strong winds prevent insects from evolving very long proboscides in certain areas, whereas lower wind speeds allow the escalatory race to proceed much further in other areas. We could also invoke differences in plant communities visited by pollinators as a factor which may affect the end point of an escalatory race (Anderson et al. 2010b).

7.4.4 Convergence of traits

7.4.4.1 Trait convergence within sites

Mutualisms usually involve many species. In multispecific mutualisms, the traits of many species may converge in response to the traits of a species (or a group of functionally similar species) that all of them are utilizing (Thompson 2005, Nuismer et al. 2013). For example, the traits of plants may converge to form a functional match in response to the traits of a shared pollinator (Figure 7.4). Groups of organisms with traits that have converged in response to a common selective force are called guilds (e.g. Faegri and van der Pijl 1979). Familiar to many is the hummingbird-pollinated guild of unscented, red-flowered plants with long corollas and exerted reproductive parts (Faegri and van der Pijl 1979), and the sweetly scented, pale, long-tubed flowers of plants pollinated by moths (Faegri and van der Pijl 1979). Many such guilds have been documented for groups of functionally similar pollinators. In some guilds, many plant species may interact with a single pollinator (Johnson 2010). Similarly, fruits also display adaptations to groups of seed dispersal vectors (van der Pijl 1982). However, we still need to question whether coevolution or unilateral evolution is the driver of trait convergence to form guilds in these asymmetrically specialized mutualisms.

Asymmetries in specialization are likely to translate into asymmetries in the reciprocity of selection: a highly specialized flower may be obligately dependent on one pollinator and consequently the pollinator may select strongly upon the floral traits of that plant. However, if that pollinator visits several plant species for nectar, it is unlikely to depend
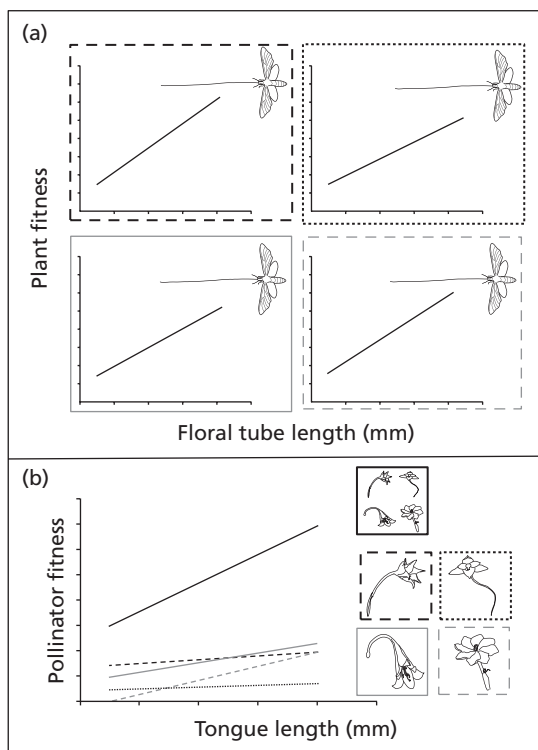
Figure 7.4 A hypothetical data set depicting the selective pressures on plant and pollinator traits (selective agents pictured for each figure) in an asymmetrically specialized pollination guild. The plant guild consists of four species: *Gladiolus longicollis* (dashed black line), *Crinum bulbispermum* (solid gray line), *Aerangis muscicola* (dotted black line), and *Gardenia thunbergia* (dashed gray line), all pollinated primarily by a single long-tongued moth pollinator, *Agrius convulvuli*. A) Because each plant is highly specialized on the moth pollinator, the moths exert a strong, positive directional selective pressure (measured by the steepness of the slope) on the tube lengths of each plant species. B) Since moths are not entirely dependent on any one plant species, each plant species will exert a relatively small selective pressure on moth proboscis length (shallow slopes). However, the cumulative effect of the entire guild (solid black line) exerts a strong selective pressure on pollinator tongue length, suggesting that coevolution in asymmetrically specialized guilds may play an important role in shaping the morphologies of all interacting partners. The relative selection strength imposed by each plant species is likely to be influenced by reward quality and quantity as well as the trait dimensions found in each population.

obligately on any one plant species; consequently, selection by one plant species on the pollinator is likely to be comparatively weak. So can such asymmetric selection lead to coevolution? Since the selection strength on a set of pollinator traits would be influenced by the entire plant guild and not just a single species, we should take into consideration the combined selective force exerted by a guild of functionally similar plant species, rather than the selection strength of each guild member individually. Since the traits of guild members often appear to be similar, each member may be generating a small but similar selective force on the pollinator. The strength of selection imposed by a guild should be strongly influenced by the similarity in the traits of the guild members. If guild members share similar traits, the combined or net selective pressure on the pollinator traits should be strong. However, if the traits of guild members are highly variable, they are likely to exert conflicting selective pressures. The resultant combined selection on the traits of the pollinator may be expected to be much weaker. In pollination guilds, the putatively coevolved traits of the floral guild members (e.g. corolla length) can show powerful convergence at the site level, such that different plants within a site are more similar to each other than are different populations of single species (Pauw et al. 2009, Anderson and Johnson 2009). This suggests that the traits of pollination guild members are frequently very similar at the site level and that they should impose similar selection pressures on the pollinator. Consequently, generalization by the pollinator is not necessarily synonymous with weak selection from the guild, because the additive selection pressures of all guild members is likely to be powerful (Figure 7.4). This way of thinking is supported by models of interacting mutualist communities, which predict that coevolution may play a very important role in the convergence and complimentarity of traits in multispecific interactions (Nuismer et al. 2013).

Due to differences in their abundance or in the quality of the commodities they provide to mutualists, some species within a guild may also play more important roles in driving coevolutionary dynamics than others. For example the traits of a rare guild member are less likely to affect the fitness of its pollinator than will a common member from the same guild, which is likely to constitute a more important food source. Nevertheless, coevolution between one interacting species pair has the ability to influence the traits of other,

non-coevolving or weakly coevolving guild members. An extreme example of this phenomenon is the coevolutionary race between the proboscides of fly pollinators and the corolla tubes of their most important nectar plants (*Zaluzianskya microsiphon*, Figure 7.2), which are closely matched at each site (Anderson and Johnson 2008). *Z. microsiphon* plants are mimicked by a rewardless orchid (*Disa nivea*) which attracts long-proboscid fly pollinators due to their close resemblance to *Z. microsiphon* (Anderson et al. 2005). Clearly, the length of the orchid's nectarless spur exerts no selection on the tongue length of the pollinator. However, because spur length affects the efficiency of pollen placement, the orchid spurs track the coevolutionary race between flies and rewarding plants. Similarly, extremely rare guild members, which exert very weak selective forces on pollinators, are also expected to track, rather than drive, coevolutionary races between pollinators and more common guild members. Consequently, if relative abundances of guild members change through space and time, the relative importance of different guild members is likely to change accordingly. As a result, different guild members may be the primary drivers of coevolution at different times or sites. In this sense Arditti et al. (2012) was probably correct in questioning the idea of coevolution between Darwin's hawkmoth and the exceptionally uncommon Madagascan Star orchid.

7.4.5 Matching of phylogenies

A common and enduring misconception about coevolution is that it should give rise to identical patterns of phylogenetic divergence (matching phylogenies) for closely interacting species (e.g. Hafner and Nadler 1988, Cuthill and Charleston 2012). Coevolution is reciprocal evolutionary change, whereas cospeciation, codiversification, and parallel cladogenesis all refer to matching between the phylogenies of interacting taxa, such that divergence events in one taxon correspond with divergences in the other (Thompson 1994). Patterns of cospeciation have been proposed across a wide range of highly specialized mutualisms (e.g. Cruaud et al. 2012, figs and wasps; Nishiguchi et al. 1998, squid and bioluminescent

bacteria; Mikheyev et al. 2010, leafcutter ants and fungi; Smith et al. 2008, yuccas and moths), as well as parasitisms (Hafner and Nadler 1988, gophers and lice; Bruyndonckx et al. 2009, bats and mites). However, almost all cophylogenies have also found host or partner switching in addition to cospeciation events. Interestingly, comparative studies on brood site mutualisms suggest that mutualists are more specialized than commensalists (Althoff et al. 2012, for interactions with yucca). Whether or not cospeciation is more prevalent in mutualist lineages than in lineages of non-pollinators that cohabit figs (Weiblen and Bush 2002) remains a matter of debate (Chapter 3). In any event, it is clear that cospeciation patterns may be observed irrespective of whether or not a relationship is coevolved (see Figure 1 in Box 7.3). In this box Kari Segraves describes how phylogenetic patterns of interacting species may be affected by patterns of gene flow which may often not be linked to coevolution, and the many ways in which phylogeny can be used to study coevolution. In fact, the parameter space for a direct causal link between coevolution and cospeciation is small because it requires cospeciation to act on the reproductive isolation of one or both species. While this is possibly the case for interactions in which one species controls or influences the mating patterns of another species, evidence for this link is limited (Moe and Weiblen 2012), or to the contrary (Althoff et al. 2012). Phylogeny is in fact a particularly poor tool for inferring most kinds of coevolution. However, it can still be a very powerful tool for coevolutionary studies. It is essential if, for example, one wishes to trace historical and contemporary patterns of host use, movement through geographic landscapes, rates of diversification, or the directions of morphological change through character state reconstructions (Chapter 3 and Box 7.3). In addition to using molecular tools to generate phylogenies, new advances in molecular technologies may enable biologists to identify the genes involved in coevolution. In Box 7.1, Noah Whiteman describes some of the methods and difficulties in identifying coevolutionary genes and how their identification may enable us to explore the links between reciprocal selection and genomic changes.


Box 7.3 Using phylogenetics to study coevolution

Kari A. Segraves

Since Ehrlich and Raven's seminal paper, coevolution has often been considered an important diversifying force in interacting taxa (Ehrlich and Raven 1964, Thompson 1994). Locally coevolving taxa may become highly specialized, potentially reducing the subsequent success of migrants moving among populations differing in coevolutionary history. This pattern of increasing specialization and reduction of gene flow among populations is the fuel for geographic variation in coevolved traits and speciation (Thompson 2005). Although coevolution may facilitate speciation in some types of interspecific interactions, recent theoretical work suggests that the direct role of coevolution in the diversification of mutualistic lineages is limited (Yoder and Nuismer 2010). Thus, there is a compelling need to rigorously assess how coevolution affects divergence in mutualistic taxa.

Phylogenetic tools offer a valuable approach to study the linkage between coevolution and diversification. Unfortunately, misinterpretation of cospeciation analyses (comparisons of the phylogenies of interacting taxa to assess the number of concordant speciation events) has incorrectly promoted the idea that matching phylogenies are sufficient evidence of coevolution (Box 7.3, Figure 1A). Phylogenetic comparisons cannot provide evidence of reciprocal evolutionary change, and like other correlative approaches, they should be combined with experimental data to demonstrate coevolution (Nuismer et al. 2010). Although concordant trees may be a result of coevolution, they may also be the result of other commonalities between two lineages, such as a shared biogeographic history that resulted in covariance (Box 7.3, Figure 1B). Thus, matching phylogenies may occur in taxa that have no coevolutionary history and, likewise, tightly coevolved taxa may have significant mismatches in speciation events (Box 7.3, Figure 1C; e.g., Segraves 2010, this chapter). Consequently, phylogenetic information in the absence of good natural history, ecological, and biogeographic data can be misleading at best, and at worst, may result in erroneous conclusions about the importance of coevolution in the speciation process.

Since coevolution cannot be inferred directly from phylogenies, what role can phylogenetics play in coevolutionary studies? A phylogenetic framework can provide the basis for testing specific hypotheses about the importance of coevolution in shaping the evolutionary histories of putatively coevolving mutualists. Carefully crafted hypotheses combined with ecological or experimental data may distinguish coevolution from alternative explanations of the
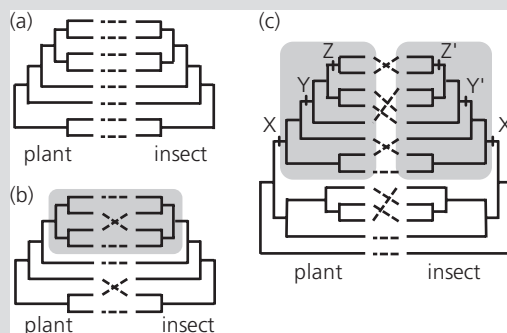
Box 7.3 Figure 1 Examples of hypothetical phylogenetic patterns resulting from cospeciation analyses of interacting plant and insect lineages. Dotted lines connecting plants and insects indicate an interaction. A) Perfect tracking and significant concordance of the insect and plant speciation events suggests strong specialization or vertical transmission; however, there is no evidence of coevolution. B) General concordance of plant and insect phylogenies indicating cospeciation with a few host shifts. Clades highlighted by the box originated at the same time as major biogeographic events, suggesting that speciation was driven by vicariance and not coevolution. C) Discordant phylogenies showing substantial host shifting. Putatively coevolved clades are indicated by the boxes and coevolved traits are shown by the letters X/X′, Y/Y′, and Z/Z′. Coevolution is suggested by correlated shifts in traits between the plant and insect lineages. There is also enhanced speciation in the coevolved clades as compared to sister groups.

phylogenetic patterns. Together, these collective datasets allow one to ask whether coevolutionary selection has led to the formation of new species (e.g., Box 7.3, Figure 1C) or if non-coevolutionary explanations are a more likely cause of divergence (e.g., Box 7.3, Figure 1B). For systems in which there are data indicating reciprocal evolutionary change, modern comparative methods can take this analysis a step further to assess whether the coevolving traits are correlated with enhanced net speciation rates (e.g., Maddison et al. 2007).

An excellent illustration of the use of phylogenetics in coevolutionary studies is the recent work on the mutualism between the charismatic yucca, the Joshua tree, and its pollinating yucca moths. The Joshua tree occurs in the North American Mojave Desert, which was historically divided by the Bouse Embayment when rising sea level extended the Sea of Cortez northward. Both the Joshua tree (Lenz 2007) and pollinator moths (Pellmyr and Segraves 2003) consist of pairs of sister species, suggesting that coevolution may be causing speciation in both plants and insects. The key

Box 7.3 *Continued*

question to address is whether diversification was driven by coevolution or vicariance caused by historical changes in sea level.

Using phylogenetic approaches, Smith et al. (2008) showed that while Joshua trees seem to have diverged concordantly with the timing of the Bouse Embayment, moth divergence is significantly more recent—so recent, that simultaneous vicariant speciation of Joshua trees and moths is highly unlikely. Floral traits important to the pollination mutualism are significantly more divergent than vegetative traits which are independent of the interaction, and differences in floral traits are also correlated with shifts in moth morphology, a pattern consistent with pollinator-mediated selection on floral traits (Godsoe et al. 2008). Ancestral state reconstruction indicates that the pollinators diversified while interacting with the Joshua tree and not due to a host shift from another yucca species. Combined, these results suggest that coevolutionary changes in traits led to reproductive isolation and speciation in both plants and insects. Although the evidence points toward coevolution as a speciation mechanism, additional ecological work is required to assess alternative explanations. For example, since the Joshua tree appears to have diversified earlier than the moths, moth divergence may have been unilaterally driven by existing morphological differences in the plant rather than by reciprocal, coevolutionary change (Smith et al. 2008).

Clearly, there are a number of important factors to consider when using phylogenetics to study coevolution. First and foremost is a need for explicit, testable phylogenetic hypotheses that distinguish coevolution from alternative explanations. Like the Joshua tree example, these hypotheses will often be taxon specific and should be tailored for the study system. Second, under no circumstance should evidence of matching phylogenies be interpreted as coevolution. More often than not, concordant phylogenies are an indicator of a non-coevolutionary interpretation. Finally,

phylogenetic studies need to be coupled with sound ecological work that shows reciprocal evolutionary change. The most powerful approach will combine clues from a diverse dataset to demonstrate a link between reproductive isolation and traits under coevolutionary selection.

References

Ehrlich, P.R., and Raven, P.H. (1964). Butterflies and plants: a study in coevolution. *Evolution*, **18**, 586–608.

Godsoe, W., Yoder, J.B., Smith, C.I., and Pellmyr, O. (2008). Coevolution and divergence in the Joshua tree/yucca moth mutualism. *American Naturalist*, **171**, 816–823.

Lenz, L.W. (2007). Reassessment of *Yucca brevifolia* and recognition of *Y. jaegariana* as a distinct species. Aliso, **24**, 97–104.

Maddison, W.P., Midford, P.E., and Otto, S.P. (2007). Estimating a binary character's effect on speciation and extinction. *Systematic Biology*, **56**, 701–710.

Nuismer, S.L., Gomulkiewicz, R., and Ridenhour, B.J. (2010). When is correlation coevolution? *American Naturalist*, **175**, 525–537.

Pellmyr, O., and Segraves, K.A. (2003). Pollinator divergence within an obligate mutualism: two yucca moth species (Lepidoptera; Prodoxidae: *Tegeticula*) on the Joshua tree (*Yucca brevifolia*; Agavaceae). *Annals of the Entomological Society of America*, **96**, 716–722.

Segraves, K.A. (2010). Branching out with coevolutionary trees. *Evolution: Education and Outreach*, **3**, 62–70.

Smith, C.I., Godsoe, W.K., Tank, S., Yoder, J.B., and Pellmyr, O. (2008). Distinguishing coevolution from covicariance in an obligate pollination mutualism: asynchronous divergence in Joshua tree and its pollinators. *Evolution*, **62**, 2676–2687.

Thompson, J.N. (1994). *The Coevolutionary Process*. Chicago, IL: University of Chicago Press.

Thompson, J.N. (2005). *The Geographic Mosaic of Coevolution*. Chicago, IL: University of Chicago Press.

Yoder, J.B., and Nuismer, S.L. (2010). When does coevolution promote diversification? *American Naturalist*, **176**, 802–817.

7.5 Conclusion

Many studies have excelled at showing patterns that could be attributed to coevolution (Thompson 2005). However, many of these patterns tend not to be mutually exclusive to the coevolutionary process. Because of this, it has been difficult to ascertain the relative importance of coevolution compared to

other evolutionary processes in driving phenotypic change. Frequently, it has been easier to reason the importance of coevolution by arguing that certain interacting organisms are codependent and that they share long, intimate evolutionary histories. From there it is possible to ask questions about putatively coevolved relationships. For example, this chapter has highlighted some of the ways in which

we expect the patterns of coevolving traits and phylogenies to differ under different interaction scenarios (e.g. the type or symmetry of the relationship). Some empirical tests of our predictions include phylogenetic data that have demonstrated that coevolution does not lead to a greater expectation of cospeciation than unilateral evolution. Similarly, mutualisms, antagonisms, and commensalisms all exhibit patterns of cospeciation. Recent studies also suggest that mutualisms and antagonisms can generate matching and mismatching of traits, although the jury is still out on whether the degree of matching/mismatching is strongly related to the kind of interaction, or whether it is related to other inherent qualities of the interacting species.

While theoreticians are hard at work formulating hypotheses for how the traits of mutualists coevolve, there remain few good empirical tests of these hypotheses. Furthermore, empirical studies on mutualisms have lagged behind work on antagonistic relationships. While it is not necessary to prove or disprove the importance of coevolution in every putative example, it is hard to gain a good understanding of coevolutionary mechanics without directly measuring the strengths and directions of selection gradients on interacting species pairs or communities of species. Although a few attempts have been made for antagonistic interactions (e.g. Benkman et al. 2003), even fewer are available for mutualisms, and measuring selection forces exerted by and received by multiple members of interaction webs is presently absent from the literature. This underlies the important question of how important coevolution is in the trait evolution of interacting communities versus interacting species pairs. While most coevolutionary models have been generated specifically for interacting pairs of species, it is evident that most mutualisms involve webs of interacting species (see Chapter 11). Future efforts need to be made in generating new models or adapting existing models on species-specific interactions so that they may be used to make predictions about how coevolution is expected to proceed in multispecific communities and what patterns we expect it to produce. So far, the expectation is that coevolution will play an important role in the evolution of traits in multispecific mutualisms (Nuismer et al. 2013).

References

Alexandersson, R., and Johnson, S.D. (2002). Pollinator mediated selection on flower-tube length in a hawk-moth pollinated *Gladiolus* (Iridaceae). *Proceedings of the Royal Society B*, **269**, 631–636.

Althoff, D.M., Segraves, K.A., Smith, C.I., Leebens-Mack, J., and Pellmyr. O. (2012). Geographic isolation trumps coevolution as a driver of yucca and yucca moth diversification. *Molecular Phylogenetics and Evolution*, **62**, 898–906.

Anderson, B., Alexandersson, R., and Johnson, S.D. (2010a). Evolution and coexistence of pollination ecotypes in an African Gladiolus (Iridaceae). *Evolution*, **64**, 960–972.

Anderson, B., and Johnson, S.D. (2008). The geographical mosaic of coevolution in a plant-pollinator mutualism. *Evolution*, **62**, 220–225.

Anderson, B., and Johnson, S.D. (2009). Geographical covariation and local convergence of flower depth in a guild of fly-pollinated plants. *New Phytologist*, **182**, 533–540.

Anderson, B., Johnson, S.D., and Carbutt, C. (2005). Exploitation of a specialized mutualism by a deceptive orchid. *American Journal of Botany*, **92**, 1342–1349.

Anderson, B., Terblanche, J.S., and Ellis, A.G. (2010b). Predictable patterns of trait mismatches between interacting plants and insects. *BMC Evolutionary Biology*, **10**, 1471–2148.

Arditti, J., Elliott, J., Kitching, I.J., and Wasserthal, L.T. (2012). "Good Heavens what insect can suck it" – Charles Darwin, *Angraecum sesquipedale* and *Xanthopan morganii praedicta*. *Botanical Journal of the Linnean Society*, **169**, 403–432.

Benkman, C.W., Parchman, T.L., Favis, A., and Siepielski, A.M. (2003). Reciprocal selection causes a coevolutionary arms race between crossbills and lodgepole pine. *American Naturalist*, **162**, 182–194.

Brouat, C., Garcia, N., Andary, C., and McKey, D. (2001). Plant lock and key: Pairwise coevolution of an exclusion filter in an ant-plant mutualism. *Proceedings of the Royal Society of London B*, **268**, 2131–2141.

Bruyndonckx, N., Dubey, S., Ruedi, M., and Christe, P. (2009). Molecular cophylogenetic relationships between European bats and their ectoparasitic mites (Acari, Spinturnicidae). *Molecular Phylogenetics and Evolution*, **51**, 227–237.

Cruaud, A., Ronsted, N., Chantarasuwan B., et al. (2012). An extreme case of plant–insect codiversification: Figs and fig-pollinating wasps. *Systematic Biology*, **61**, 1029–1047.

Cuthill, J.H., and Charleston, M. (2012). Phylogenetic co-divergence supports coevolution of mimetic *Heliconius* butterflies. *PLoS ONE*, **7**, e36464.

Darwin, C.R. (1862). *On the Various Contrivances By which British and Foreign Orchids Are Fertilized by Insects*, facsimile edn. London: John Murray.

Decaestecker, E., Gaba, E., Raeymaekers, J.A.M., Stoks, R., Van Kerckhoven, L., Ebert, D., and De Meester, L. (2007). Host–parasite "Red Queen" dynamics archived in pond sediment. *Nature*, **450**, 870–874.

Ehrlich, P.R., and Raven, P.H. (1964). Butterflies and plants: a study in coevolution. *Evolution*, **18**, 586–608.

Ellis, A.G., and Johnson, S.D. (2010). Gender differences in the effects of floral spur length manipulation on fitness in a hermaphrodite orchid. *International Journal of Plant Sciences*, **3**, 1010–1019.

Faegri, K., and van der Pijl, L. (1979). *The Principles of Pollination Ecology*, 3rd rev edn. Oxford: Pergamon Press.

Flor, H.H. (1942). Inheritance of pathogenicity in *Melampsora lini*. *Phytopathology*, **32**, 653–669.

Galetti, M., Guevara, R., Côrtes, M.C., et al. (2013). Functional extinction of birds drives rapid evolutionary changes in seed size. *Science*, **340**, 1086–1090.

Gandon, S., Capowiez, Y., Dubois, Y., Michalakis, Y., and Olivieri, I. (1996). Local adaptation and gene-for-gene coevolution in a metapopulation model. *Proceedings of the Royal Society of London B*, **263**, 1003–1009.

Geerts, S., and Pauw A. (2009). African sunbirds hover to pollinate an invasive hummingbird-pollinated plant. *Oikos*, **118**, 573–579.

Geffeney, S.L., Fujimoto, E., Brodie, E.D. III, Brodie, E.D. Jr., and Ruben, P.C. (2005). Evolutionary diversification of TTX-resistant sodium channels in a predator-prey interaction. *Nature*, **434**, 759–763.

Grant, P.R., and Grant, R.B. (2006). Evolution of character displacement in Darwin's finches. *Science*, **313**, 224–226.

Guimarães, P.R., Jordano, P., and Thompson, J.R. (2011). Evolution and coevolution in mutualistic networks. *Ecology Letters*, **14**, 877–885.

Hafner, M.S., and Nadler, S.A. (1988). Phylogenetic trees support the coevolution of parasites and their hosts. *Nature*, **332**, 258–259.

Hanifin, C.T., Brodie, E.D. Jr., and Brodie, E.D. II. (2008). Phenotypic mismatches reveal escape from arms-race coevolution. *PLoS Biology*, **6**, 471–482.

Howe, H.F. (1984). Constraints on the evolution of mutualisms. *American Naturalist*, **123**, 764–777.

Janzen, D.H. (1980). When is it coevolution? *Evolution*, **34**, 611–612.

Janzen, D.H. (1981). Patterns of herbivory in a tropical deciduous forest. *Biotropica*, **13**, 271–282.

Johnson, S.D. (2010). The pollination niche and its role in the diversification and maintenance of the southern African flora. *Philosophical Transactions of the Royal Society*, **365**, 499–516.

Johnson, S.D., Hargreaves, A.L., and Brown, M. (2006). Dark, bitter-tasting nectar functions as a filter of flower visitors in a bird-pollinated plant. *Ecology*, **87**, 2709–2716.

Koskella, B., and Lively, C.M. (2007). Advice of the rose: experimental coevolution of a trematode parasite and its snail host. *Evolution*, **61**, 152–159.

Lodwig, E.M., Hosie, A.H.F., Bourdès, A., et al. (2003). Amino-acid cycling drives nitrogen fixation in the legume-Rhizobium symbiosis. *Nature*, **422**, 722–726.

Mikheyev, A.S., Mueller, U.G., and Abbott, P. (2010). Comparative dating of attine ant and lepiotaceous cultivar phylogenies reveals coevolutionary synchrony and discord. *American Naturalist*, **175**, E126–E133.

Miklasheviches, E., Röhrig, H., Schell, J., and Schmidt, J. (2001). Perception and signal transduction of rhizobial NOD factors. *Critical Reviews in Plant Sciences*, **20**, 373–394.

Mode, C.J. (1958). A mathematical model for the coevolution of obligate parasites and their hosts. *Evolution*, **12**, 158–165.

Moe, A.M., and Weiblen, G.D. (2012). Pollinator-mediated reproductive isolation among dioecious fig species (Ficus, Moraceae). *Evolution*, **66**, 3710–3721.

Muchhala, N., and Thomson, J.D. (2009). Going to great lengths: selection for long corolla tubes in an extremely specialized bat-flower mutualism. *Proceedings of the Royal Society B*, **276**, 2147–2152.

Nattero, J., and Cocucci, A.A. (2007). Geographical variation in floral traits of the tree tobacco in relation to its hummingbird pollinator fauna. *Biological Journal of the Linnean Society*, **90**, 657–667.

Newman, E., Manning, J., and Anderson, B. (2014). Fine scale syndrome dynamics within a guild: geographic trait matching between long proboscid flies and the flowers that they pollinate. *Annals of Botany*, **113**, 373–384.

Nilsson, L.A. (1988). The evolution of flowers with deep corolla tubes. *Nature*, **334**, 147–149.

Nishiguchi, M.K., Ruby, E.G., and McFall-Ngai, M.J. (1998). Competitive dominance among strains of luminous bacteria provides an unusual form of evidence for parallel evolution in sepiolid squid-vibrio symbioses. *Applied and Environmental Microbiology*, **64**, 3209–3213.

130 MUTUALISM

Nuismer, S.L., Gomulkiewicz, R., and Ridenhour, B.J. (2010). When is correlation coevolution? *American Naturalist*, **175**, 525–537.

Nuismer, S.L., Jordano, P., and Bascompte, J. (2013). Coevolution and the architecture of mutualistic networks. *Evolution*, **67**, 338–354.

Nuismer, S.L., Thompson, J.N., and Gomulkiewicz, R. (1999). Gene flow and geographically structured coevolution. *Proceedings of the Royal Society of London B*, **266**, 605–609.

Pauw, A., Stofberg, J., and Waterman, R.J. (2009). Flies and flowers in Darwin's race. *Evolution*, **63**, 268–279.

Pellmyr, O., and Thompson, J.N. (1996). Sources of variation in pollinator contribution within a guild: the effects of plant and pollinator factors. *Oecologia*, **107**, 595–604.

Rich, K.A., Thompson, J.N., and Fernandez, C.C. (2008) Diverse historical processes shape deep phylogeographic divergence in a pollinating seed parasite. *Molecular Ecology*, **17**, 2430–2448.

Schemske, D.W., and Horwitz, C.C. (1984). Variation among floral visitors in pollination ability: a precondition for mutualism specialization. *Science*, **225**, 519–521.

Smith, C. I., Godsoe, W.K.W., Tank, S., Yoder, J.B., and Pellmyr, O. (2008). Distinguishing coevolution from covariance in an obligate pollination mutualism: asynchronous divergence in Joshua tree and its pollinators. *Evolution*, **62**, 2676–2687.

Stebbins, L. (1970). Adaptive radiation of reproductive characteristics in angiosperms. I. Pollination mechanisms. *Annual Review of Ecology and Systematics*, **1**, 307–326.

Steiner, K.E., and Whitehead, V.B. (1991). Oil flowers and oil bees: further evidence for pollinator adaptation. *Evolution*, **45**, 1493–1501.

Temeles, E.J., and Kress, W.J. (2003). Adaptation in a hummingbird association. *Science*, **300**, 630–633.

Temeles, E.J., Pan, I.L., Brennan, J.L., and Hotwitt, J.N. (2000). Evidence for ecological causation of sexual dimorphism in a hummingbird. *Science*, **289**, 441–443.

Thompson, J.N, (1989). Concepts of coevolution. *Trends in Ecology and Evolution*, **4**, 179–183.

Thompson, J.N. (1994). *The Coevolutionary Process*. Chicago: The University of Chicago Press.

Thompson, J.N. (1997). Evaluating the dynamics of coevolution among geographically structured populations. *Ecology*, **78**, 1619–1623.

Thompson, J.N. (1999). Specific hypotheses on the geographic mosaic of coevolution. *American Naturalist*, **153**, S1–S14.

Thompson, J.N. (2005). *The Geographic Mosaic of Coevolution*. Chicago: The University of Chicago Press.

Thompson, J.N. (2013). *Relentless Evolution*. Chicago: The University of Chicago Press.

Thompson, J.N., and Cunningham, B.M. (2002). Geographic structure and dynamics of coevolutionary selection. *Nature*, **417**, 735–738.

Thompson, J.N., and Pellmyr, O. (1992). Mutualism with pollinating seed parasites amid co-pollinators: constraints on specialization. *Ecology*, **73**, 1780–1791.

Thompson, J.N., Schwind, C., Guimarães, G.B Jr., and Friberg M (2013). Diversification through multitrait evolution in a coevolving interaction. *Proceedings of the National Academy of Sciences*, **110**, 11487–11492.

Thrall, P.H., and Burdon, J.J. (2003). Evolution of virulence in a plant host-pathogen metapopulation. *Science*, **299**, 1735–1737.

Thrall, P.H., Laine, A-L., Ravensdale, M., Nemri, A., Dodds, P.N., Barrett, L.G., and Burdon, J.J. (2012). Rapid genetic change underpins antagonistic coevolution in a natural host-pathogen metapopulation. *Ecology Letters*, **15**, 425–435.

Toju, H., Abe, H., Ueno, S., Miyazawa, Y., Taniguchi, F., Sota, T., and Yahara, T. (2011). Gradients of arms race coevolution. *American Naturalist*, **177**, 562–573.

Toju, H., and Sota, T. (2006). Imbalance of predator and prey armament: Geographic clines in phenotypic interface and natural selection. *American Naturalist*, **167**, 105–117.

van der Pijl, L. (1982). *Principles of Dispersal in Higher Plants*. New York: Springer-Verlag.

van Noort, S., and Compton, S.G. (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.

Wasserthal, L.T. (1997). The pollinators of the Malagasy star orchids *Angraecum sesquipedale, A. sororium*, and *A. compactum* and the evolution of extremely long spurs by pollinator shift. *Botanica Acta*, **110**, 343–359.

Weeks, A.R., Turelli, M., Harcombe, W.R., Reynolds, T., and Hoffmann, A.A. (2007). From parasite to mutualist: rapid evolution of *Wolbachia* in natural populations of *Drosophila*. *PloS Biology*, **5**, e114.

Weiblen, G.D. (2004). Correlated evolution in fig pollination. *Systematic Biology*, **53**, 128–139.

Weiblen, G.D., and Bush, G.L. (2002). Speciation in fig pollinators and parasites. *Molecular Ecology*, **11**, 1573–1578.

Yoder, J.B., and Nuismer, S.L. (2010). When does coevolution promote diversification? *American Naturalist*, **176**, 802–817.