Qualifying Exam

My Answers

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Pollination efficiency

Interest in pollination efficiency began with Sprengel's inception of pollination biology in 1793. With his discovery of the importance of animal pollination, Sprengel marveled at how well flowers were constructed to effect pollen transfer from one plant to another (Vogel, 1996). Darwin (1862) further championed this interpretation of pollination efficiency in his examination of orchids; although natural selection rather than divinity was now given credit for the curious contrivances he observed. Under this view aspects of floral morphology were interpreted as enhancing the export and receipt of pollen (i.e., pollination efficiency). However, this focus shifted slightly after Darwin's investigations into the effects of cross and self-fertilisation (Darwin, 1876). With the benefits of avoiding self-fertilisation established (see Charlesworth and Charlesworth, 1987; Husband and Schemske, 1996, for modern views), floral characters were viewed as anti-selfing mechanisms. (The subtle differences between ensuring cross-fertilisation and preventing self-fertilisation will be explained below.) The work of David Lloyd has largely been responsible for the renewed interest in the efficiency of pollination, as is illustrated by the following excerpt (Lloyd, 1984, emphasis in original):

> Intrasexual selection to increase the proficiency of pollen donation, *particularly the number of visitors that can remove pollen from a flower*, is the major selective force guiding floral evolution

This discussion will focus on the consequences of the reemphasis on pollen donation and receipt for our understanding of floral biology. I will begin with a brief review of the distinction between promoting outcrossing and preventing selfing. This will be followed by a formulation of pollination efficiency. Lastly, the influence of floral biology on pollination efficiency will be discussed. If pollination efficiency is a target for selection, the form and function of flowers should reflect this selective pressure.

Cross-fertilisation vs. inbreeding avoidance

To address the subtle distinction between preventing selfing and promoting outcrossing, every hermaphroditic plant achieves reproductive success through both male and female function. Bateman's principle (Bateman, 1948) essentially states that female function is limited by access to resources while male function is limited by access to ovules. Stated another way, females are limited by offspring quality and males by offspring quantity. As a result, any mechanism that impedes selfing benefits female function while male function benefits from mechanisms that promote outcrossing. The asymmetric effect of outcrossing on male reproductive success has led many to reinterpret the evolution of floral structures as primarily benefiting male function (e.g., Sutherland and Delph, 1984; Bell, 1985). Perhaps the most beneficial effect of this reinterpretation is that now, instead of just female function, *both* male and female functions are considered important in the evolution of floral biology.

A definition of pollination efficiency

Pollination efficiency may seem intuitive as a concept. However, intuition often does not translate well into specific definitions and applications. Stripped to its fundamentals pollination consists of removal of pollen from anthers and subsequent deposition on stigmas. As such, a potential definition of pollination efficiency could be the ratio of pollen receipt to pollen deposition following the visit of a single pollinator. This definition has the virtues of being operational and analytically simple, with the vice of subsuming a great deal of biological diversity into a single number. At the very least, this definition can be used as an heuristic device to examine the factors that influence pollination.

To explicate this definition further, consider the pollination process illustrated in Figure 1.1 (following Harder and Thomson, 1989)[1] . In this example a bee devoid of pollen visits a flower that contains A pollen grains and removes R of these pollen grains. It subsequently visits another flower and deposits D of the R grains it carries. Given this simple scenario, an estimate of pollination efficiency (ϵ) would be D/R. Given that the pollinator initially does not carry any pollen, it follows that D<R. Consequently, $0<\epsilon<1$. Approaches for maximising ϵ

[1]There are many theoretical models for pollen transfer, each with its own set of symbols (e.g., Holsinger and Thomson, 1994; Rademaker *et al.*, 1997). I have chosen to follow Harder and Thomson (1989) for simplicity

are either increase D, reduce R, or both. This matches our intuition that efficient pollination should result in most of the pollen removed being deposited on a stigma.

The parameters D and R can be estimated from both lab and field studies. In the lab bumble-bees can be induced to visit flowers in sequence (Rademaker *et al.*, 1997). Calculating the difference between pollen counts from anthers collected prior and subsequent to bee visitation would estimate R (Harder and Thomson, 1989). Harvesting stigmas after visitation and counting their pollen loads would provide D. In a study of this nature, the experience of pollinators may be important (Laverty, 1994). When bees first encounter a new type of flower, they spend a significant amount of time learning the best strategies for accessing pollen and nectar (this can take 20-60 minutes). As a consequence, inexperienced bees may produce estimates of pollination efficiency very different from experienced bees. To mitigate this difficulty, bees can be trained on emasculated flowers. This way the bees learn how to approach and manipulate the flowers, while remaining free of contaminant pollen that would bias the estimate of ϵ. Field studies present the difficulty of finding pollinators without pollen loads. One approach to this problem is the use of coloured dyes as pollen analogues (Campbell, 1989a,b). The dye can be added to the pollen of a focal plant and then subsequent deposition of the dye at another flower visited can be used to estimate D. As with the lab studies, comparison of anthers before and after visitation can provide R.

As a concrete example, consider the work of Rademaker *et al.* (1997). In this experiment a green fluorescent dye was applied to the anthers of a single *Echium vulgare* flower and then presented to a foraging bumble-bee. After visiting the dyed flower the bumble-bee was then presented with an artificial plant containing 32 emasculated flowers. The visitation pattern of the bumble-bee was noted and the styles of visited flowers were collected for quantifying dye deposition. The movement of dye relative to pollen has been well characterised in this system, so Rademaker et al. were able to estimate pollen deposition rates directly from dye deposition. To estimate the amount of pollen removed by a single visit, stamens from unvisited flowers were compared to those from visited flowers. Any differences between the two were attributed to removal by the pollinator. To summarise the results, an anther contains on average 80,000 pollen grains before visitation of which approximately 13,000 are removed by a single visit. A subsequent visit by the bee to another flower deposited only 9 pollen grains on average. This would yield an estimation of $\epsilon = 9/13{,}000 = 0.0007$. To place this result in context, *Erythronium* flowers have an ϵ of 0.006 (Harder and Thomson, 1989) and butterfly-pollinated

Phlox flowers had an ϵ of 0.005 (Levin and Berube, 1972). Of course, in evolutionary terms everything is relative. A species with, what seem to us, extremely low ϵ values will still be selected for if its competition is even less efficient. Nonetheless, if floral biology can modify ϵ, how might selection improve on these efficiency measures?

Packaging and dispensing mechanisms

One important insight gained from the Rademaker *et al.* (1997) study is that just over half of any pollen removed from anthers is lost during either foraging or traveling between flowers. This incredible wastage of pollen is essentially dropped to the ground by the pollinator and lost from the system. Moreover, Harder and Thomson (1989) demonstrated that the amount of pollen deposited on stigmas (D) is a rapidly decelerating function of the number of pollen grains removed (R). This means that a flower exporting small amounts of pollen will have a proportionally larger amount deposited than will a flower that exports large amounts of pollen. By spreading their pollen production into small packages over a wide number of pollinators, plants should be able to maximise their pollen deposition on stigmas. Plants can accomplish this through either packaging or dispensing mechanisms (Lloyd and Yates, 1982). In packaging, a plant's total pollen production is divided into discrete units and revealed to pollinators over time. Dispensing mechanisms restrict the amount of pollen that can be removed from a package of pollen with each visit (reviewed in Harder and Thomson, 1989).

These packaging and dispensing mechanisms can be interpreted as ways to restrict the amount of pollen removed with each visit by a pollinator. This reduces the absolute amount of pollen wasted in the process and, thereby, increases the amount of pollen available for deposition while decreasing the amount removed. Interpreted as a response to selection for pollination efficiency, this would enhance the value of ϵ measured for pollen packaging and dispensing species. As such, selection appears to be maximising ϵ. However, as an alternate explanation, Lloyd and Yates (1982) demonstrated that competition between plants for access to receptive stigmas (male intrasexual competition) can also favour the lengthening of male phase provided by these dispensing mechanisms. This alternate explanation is only valid in cases where seed set is not limited by pollen availability. Consequently, experimental tests of pollen limitation could be used to evaluate these two hypotheses for the evolution of pollen packaging and dispensing.

Floral design

Pollinators are certainly influenced by the size and shape of flowers. Herrera (1987, 1989) has characterised this influence as affecting either pollinator quantity or quality. Pollinator quantity is a measure of the frequency with which pollinators visit a flower. Pollinator quality refers to the amount of pollen removed and deposited by visitors and is equivalent to the concept of pollination efficiency described here. The question is, can floral design specifically influence pollinator quality?

Stanton *et al.* (1986) demonstrated the influence of flower colour on pollination in *Raphanus raphanistrum*. *R. raphanistrum* has a petal colour polymorphism with both white- and yelow-petalled morphs occurring naturally. Individuals of both morphs were placed into experimental arrays of plants and the colour of the resulting progeny was used to infer male reproductive success. Although seed set was unaffected by petal colour, yellow-petalled flowers donated most of the pollen in the arrays. This could be interpreted as yellow-petalled flowers having a higher ϵ than white-petalled flowers. However, it is difficult to imagine a mechanism for petal colour influencing pollination efficiency. The more likely explanation, indeed the conclusion of the Stanton et al. study, is that pollinators were attracted by the yellow colouring and, therefore, visited yellow-petalled flowers more often (i.e., pollinator quantity dominates). This example demonstrates the importance of the distinction made by Herrera between quality and quantity. Since they can have superficially similar effects, it is not wise to assume that pollination efficiency provides the selective pressure for floral appearances.

Campbell (1996) found a positive relationship between flower width and the removal of pollen in *Ipomopsis aggregata*. However, there was no evidence for a similar correlation between flower width and pollen deposition. Similarly, Young and Stanton (1990) found a positive correlation between corolla size and pollen removal, but not pollen deposition, for *Raphanus sativus*. Since D is not influenced by width, if selection favours high ϵ values in these species, flower width should decrease over time to reduce R. However, there is no evidence for a decrease in flower size in these species. In a review of the literature, Wilson *et al.* (1994) state that, at best, there is a weak relationship between floral morphology and R and D. This implies that pollination efficiency cannot be modified by floral morphology.

Campbell (1996) summarise this research by pointing out that little direct evidence demonstrates that floral morphology affects pollination efficiency. They suggest that there

is much better evidence that morphological features influence a pollinator's ability to access floral rewards (particularly nectar) and that pollinators learn to visit these rewarding flowers more frequently. Consequently, pollinator quantity may be much more important than quality in explaining floral evolution. Furthermore, Herrera (1993) found a significant influence of floral morphology on pollen deposition rates. However, this effect was dwarfed by ecological factors that had a much larger effect on plant fitness. Taken as a whole, these results suggest that floral morphology is not evolving in response to pollination efficiency.

Nectar rewards

Pollinators do not visit flowers to facilitate fertilisation. Rather, they have their own selfish reasons; primarily collecting nectar (Proctor *et al.*, 1996). Since many of these pollinators are dependent on nectar for survival, plants have a potent source of control over pollinator behaviour. If pollination efficiency is favoured by selection, we might expect nectar quality and quantity to be modified to enhance ϵ. Galen and Plowright (1985) experimentally manipulated nectar availability in flowers of *Chamerion angustifolium* and observed the reactions of bumble-bees to this change. Bumble-bees tended to spend more time visiting nectar-enriched flowers and, consequently, deposited more pollen. Similar manipulations of hawkmoth-pollinated *Mirabilis multiflora* nectar found an increase in both pollen removal and deposition, largely due to an increase in visitation rates (Hodges, 1995). Furthermore, Aizen and Basilio (1998) found that variation in nectar availability in *Alstroemeria aurea* influenced the frequency of bee visitation, but not R or D. As with floral morphology, nectar availability appears to be used as a mechanism for modifying pollinator quantity rather than quality.

Gender positioning

Another aspect of floral morphology that could influence pollination efficiency is the positioning of the anthers and stigma. The majority of angiosperms are cosexual, possessing both male and female sexual structures in the same flower (Lloyd and Yates, 1982). Successful pollination requires that a pollinator transfer pollen from anther to stigma. As a consequence, in many hermaphroditic species both stigmas and anthers have to be presented in the path of

pollen movement. However, this overlap may interfere with pollen dispersal and receipt, reducing pollination efficiency. This interference has various manifestations. If pollinator activity transfers pollen from anther to stigma within a flower, this transfer can interfere with outcross pollen by restricting its deposition, displacing it from the stigma, or diluting it with copious self-pollen (Lloyd and Webb, 1986). This "stigma clogging" may also damage the surface of the stigma (van der Pijl, 1978) or hinder the growth of pollen tubes (Howlett *et al.*, 1975). In addition, if the stamens restrict access to the stigma they may remove incoming pollen from a pollinator's body. Conversely, the stigma may impede a pollinator's access to the stamens or remove outgoing pollen. Each of these effects modify D and R, causing a reduction in ϵ. Therefore, any adaptations that reduce interference could enhance pollination efficiency.

In many species, the close physical proximity of anthers and stigmas effectively ensures that interference will occur. However, perhaps due to the negative consequences of interference, several mechanisms have evolved to separate gender functions. Dichogamy (the separation of genders in time within a flower) and herkogamy (separation in space) have both been interpreted as mechanisms for avoiding interference (Lloyd and Webb, 1986; Webb and Lloyd, 1986). Gender has also been segregated into separate flowers in an impressive array of different breeding systems (Box 1; Richards, 1986). Bawa and Opler (1975) and Bawa (1980) suggested that unisexuality evolved for the same reason as dichogamy and herkogamy, to prevent interference between genders. However, Lloyd (1982) argued that the avoidance of self-fertilisation was a more likely explanation for the separation of gender into different individuals. He presented two pieces of evidence in support of his conclusion, both of which hinge on the asymmetrical impact of inbreeding on female reproductive success. First, gynodioecy largely predominates over androdioecy. This prevalence of female individuals suggests that females benefit disproportionately from unisexuality. Second, unisexuality is more common in long-lived, woody species. Longevity enhances the negative consequences of inbreeding (Morgan *et al.*, 1997) and, as a result, the evolution of separate sexes would be favoured to reduce inbreeding. Unlike dichogamy and herkogamy, the evolution of unisexuality is favoured by inbreeding avoidance, rather than pollination efficiency.

In this case, substituting the term "pollination efficiency" for "interference avoidance" seems to be of dubious benefit. Interference avoidance is well established in the literature and better describes the actual cause of the selective pressure for the within-flower gender separation. Perhaps, if pollination efficiency provides a general description for selection on

floral biology, interference avoidance could be considered a special case of the more general selective pressure to maximise ϵ. However, research to date does not support the universal application of pollination efficiency.

Multi-flowered inflorescences

This discussion has been framed in the procrustean condition of single flowered plants. However, many species of angiosperms display more than one flower simultaneously. Perhaps the most important consequence for multi-flowered inflorescences is geitonogamy (the transfer of pollen between flowers of the same individual; Lloyd and Schoen, 1992; de Jong *et al.*, 1993). Geitonogamy has many characteristics of outcrossing: pollinator attraction and visitation; and the removal, transfer, and deposition of pollen. However, since the pollen transfer occurs within the individual plant, geitonogamy carries the costs of inbreeding depression (Charlesworth and Charlesworth, 1987; Husband and Schemske, 1996). Nonetheless, larger inflorescences attract more pollinators (Schemske, 1980; Queller, 1983; Schmid-Hempel and Speiser, 1988). This causes an attraction paradox (Holsinger, 1996). Large floral displays attract more pollinators, but most of the pollen movement occurs within the plant (Harder and Barrett, 1995). Consequently, the evolution of floral display size may represent a compromise between maximising pollinator visitation, while minimising geitonogamy. Notice that this is once again an issue of pollinator quantity, where the number of visitors received by a plant is providing the selective pressure for changes in inflorescence size. However, viewed from the interference-avoidance perspective, geitonogamy is a consequence of female function (the stigma of another flower within the plant) interfering with male function (pollen export beyond the plant). As for within-flower interference, this between-flower interference can be viewed as reducing pollination efficiency, but the interference terminology is descriptive and well established.

These concerns aside, ϵ can be modified to include multiple flowers. Pollen exported within a plant should not represent an efficient process. To account for this, the level of analysis can be raised from the floral level to the level of the inflorescence. An inflorescence-level measure of pollination efficiency could be calculated as the ratio of pollen exported to stigmas of different plants to the total amount of pollen removed from the plant's anthers. Since a proportion of the removed pollen does not leave the plant, geitonogamy would cause a reduction in the inflorescence level measure of pollination efficiency (ϵ_i). Efficiency could be measured in a

hierarchical manner, with estimates for floral (ϵ_f) and inflorescence (ϵ_i) efficiencies calculated and compared for different species.

Alternative synthesis

> Pollen removal and pollen receipt are separate processes, and structures or behaviors that are optimal for one process do not always coincide precisely with those that are optimal for the other
> Lloyd and Yates 1982

Earlier, pollination was described as pollen removal and pollen deposition. The assumption underlying pollination efficiency is that these two processes should be closely tied together. The quote above and the discussion here, suggest that this is not the case. In terms of pollen loads, male and female function have vastly different requirements. Cruden (1977) estimated that maximum seed set typically requires pollen loads of 2-7 grains per ovule. However, pollen production and removal appears excessive given these relatively low requirements for complete fertilisation. Possible reasons for this discrepancy were discussed earlier. First of all, a large portion of the pollen removed from an anther is lost during transport (Rademaker *et al.*, 1997). Since this is largely a result of pollinator behaviour, there is little scope for floral biology to influence this source of loss. The only response available appears to be packaging and dispensing of pollen into small amounts spread over time (Harder and Thomson, 1989). This ensures that, although a large proportion of each pollen load is lost, the absolute amount of pollen available for fertilisation is maximised both in space and time. Another reason for the overproduction of pollen could be competition between pollen grains for access to unfertilised ovules (Willson, 1994). In some respects, stigmas can be viewed as randomly sampling from the pool of pollen available in the population. In this case, the number of pollen grains that an individual contributes to fertilisation is a direct product of its pollen production. Consequently, the reproductive strategy that confers the greatest success as a male would be to produce as much pollen as possible. This male-male competition could result in sexual selection for increased pollen production, regardless of the requirements for female function (the pollen-donation hypothesis; Queller, 1983; Broyles and Wyatt, 1990b,a). Efficiency does not characterise the results of such sexual selection. In fact, Darwin (1871) invoked sexual selection to explain examples of obvious

inefficiencies and extravagances in biology. Furthermore, since pollen is cheap to produce (relative to ovule and seed production) there is little incentive for efficiency in its production and dissemination. The evidence presented earlier suggests that much of floral biology is devoted to pollinator attraction (Herrera's pollinator quantity). This, coupled with the dispensing of pollen over time, ensures that each individual plant maximises its representation in the pollen pool and fertilises as many ovules as possible.

Conclusions

Pollination is not an efficient process in the sense of maximal pollen deposition for a minimal investment in pollen production. In fact, pollinator attraction and male-male competition appear to govern much of the evolution of floral biology. However, this evaluation of the operational definition of pollination efficiency does not necessarily include the concept of efficiency in pollination biology. The application of optimality models and other techniques inherent to efficiency concepts still provide valuable insights into pollination. The main disadvantage of the definition of pollination efficiency used here was that too much biological reality was lost in the translation to ϵ. The tenuous connection between pollen donation and receipt and the different requirements of the two genders makes efficiency, at best, a subsidiary goal of floral evolution.

Box 1 Plant sexual systems occur in a wide variety of types. They are listed below with their approximate frequency of occurrence (Richards, 1986).

1. sexually monomorphic systems
   (a) perfect flowered hermaphroditism (72%)
   (b) monoecy (5%): cosexual individuals, unisexual flowers
   (c) andromonoecy (rare): cosexual individuals, cosexual & male flowers
   (d) gynomonoecy (3%): cosexual individuals, cosexual & female flowers
2. sexually dimorphic systems
   (a) gynodioecy (7%): cosexual & female individuals, cosexual/female flowers
   (b) androdioecy (rare): cosexual & male individuals, cosexual/male flowers
   (c) dioecy (4%): unisexual individuals, unisexual flowers

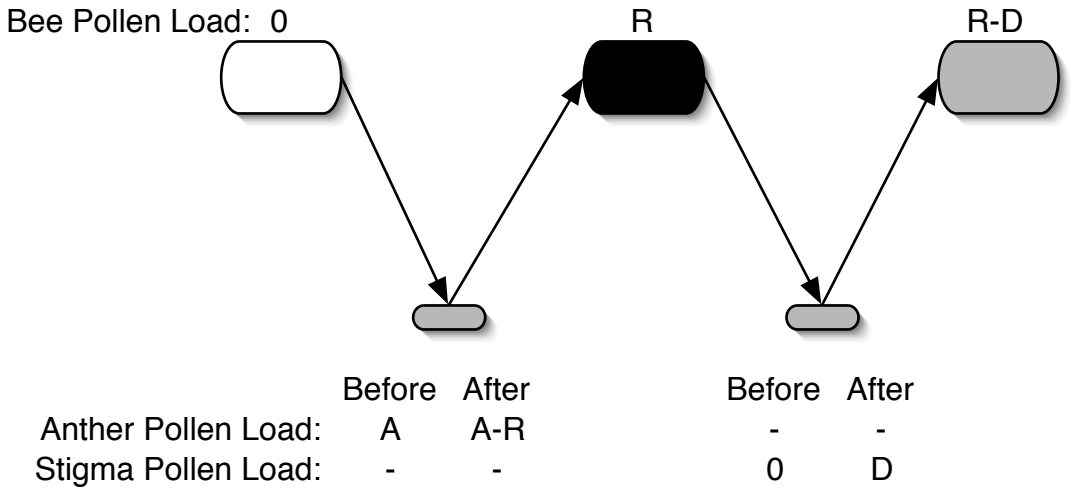

Figure 1: A diagrammatic representation of pollination. The pollen load carried by a bee is described at the top of the figure and flower pollen loads are described at the base. Dashes represent extraneous values for this model. They could be included in a more complex model of pollen carryover between flowers.

Molecules and the comparative method

Traditionally, the comparative method has been unfairly characterised as inferior to the experimental method (Mayr, 1982, Chapter 2). However, since its inception evolutionary biology has benefited greatly from comparing groups of species (e.g., Darwin, 1859). Molecular systematics has caused a modern renaissance of the comparative method and has proven extremely valuable as a research tool. I will argue that the source of this value is in molecular systematic's ability to generate accurate and accessible phylogenies. To support this argument I will demonstrate how phylogenies are used to solve some of the more difficult issues in the study of adaptation. Many of these issues are conceptually unrelated. However, their common resolution is found in the historical context that molecular systematics provides.

Phylogenetic trees

Since evolution operates through descent with modification, determining relationships between species is essential for understanding their biology. Historically, these genealogical relationships were reconstructed with morphological data. Recently, however, molecular data has begun to supplant morphology in phylogeny reconstruction (see Crawford 1990; Hillis *et al.* 1996, for reviews of molecular techniques; Patterson *et al.* 1993, for a discussion of molecules vs. morphology). The evolutionary history of a lineage leaves its mark on the genome. Consequently, differences in genome structure and composition can be used as a record of evolutionary change. Compared to the restricted set of morphological characters available, the genome has the advantage of near limitless character variation at several levels of organisation (e.g., base pair composition, insertions and deletions, restriction fragments). In addition, different sections of the genome change at different rates. This allows a choice of molecular markers to suit the taxonomic level under study. Slowly evolving pieces of DNA can be used for inves-

tigating ancient evolutionary events, while faster evolving sections are appropriate for recent events. As with any research technique, molecular systematics has its own complexities and subtleties. However, all things considered, molecular techniques allow researchers to confidently infer phylogenetic relationships relatively easily and quickly. This facilitation of systematics has transformed phylogenetic reconstruction from an intricate discipline into a powerful tool available to almost any interested biologist. As a result, phylogenies are being applied to many different biological questions. This has been particularly fruitful in the study of adaptation.

Adaptation

The investigation of the causes and consequences of adaptation is perhaps the premiere activity of evolutionary biology. The actual definition of an adaptation is the subject of much debate (West-Eberhard, 1992). However, there is some consensus that an adaptation is a characteristic of an organism whose form is the result of selection in a particular functional context (Williams, 1966; Futuyma, 1998). This definition recognises that adaptations are strongly tied to history (Gould and Vrba, 1982). In particular, the putative adaptation should be compared to the ancestral state from which it arose. To qualify as an adaptation the novel characteristic must enhance fitness relative to this ancestral state. This presents the rather difficult problem of reconstructing the past. The fossil record may provide evidence of ancestral conditions that can be evaluated for fitness. However, fossil evidence is rare (particularly for floral morphology) and does not record the ecological context required to properly evaluate fitness (Jablonski *et al.*, 1986). This has led some researchers to opt for the less satisfying ahistorical definition of adaptation as current utility, regardless of the trait's origin (Burian, 1992; West-Eberhard, 1992). Unfortunately, this definition ignores the fact that adaptations are shaped by history and often confuses the causes of adaptations with their effects (Gould and Vrba, 1982).

The construction of phylogenies permits the inference of both ancestral character states and past selective conditions (Baum and Larson, 1991; Harvey and Pagel, 1991; Maddison and Maddison, 1992). This allows the proper historical definition of adaptation to be used in a rigorous fashion. A comparison of Figures 2.1 and 2.2 demonstrates the basic argument. Suppose we wish to test the hypothesis that self-fertilisation in plants is an adaptation for reproductive assurance in an ephemeral annual lifestyle (this contrived example is inspired by Barrett *et al.*, 1996). Both figures present the same phylogenetic tree for four taxa (labeled A

through D) with their associated life-history and mating-system values. Figure 2.1 is consistent with the stated hypothesis. The mapping of the character states onto the tree suggests that the annual habit evolved in the common ancestor of taxa C and D and that selfing evolved in taxon D in response to this shift in life history. Of course a proper evaluation of the hypothesis would involve many more species and evolutionary transitions (see Barrett *et al.*, 1996), but this should suffice for illustrative purposes. Conversely, Figure 2.2 is inconsistent with the hypothesis for the evolution of selfing. In this case selfing precedes the life history change, suggesting that the annual habit was not the selective pressure that gave rise to selfing.

This technique provides a powerful tool for investigating character evolution and places adaptation into its proper historical context. However, it has only just begun to reach a prominent position in the plant literature. Botanists have certainly been cognisant of the importance of incorporating phylogeny into their investigations of plant adaptations. Important comparative analyses, such as Schemske and Lande's (1985) investigation of mating system diversity and Bertin's (1993) study of dichogamy, discussed the implications of taxonomic dependence on their results. However, these researchers did not have access to a suitable phylogeny. With the recent publication of a phylogeny for the families of the angiosperms (Chase *et al.*, 1993; Soltis *et al.*, 1999), investigations of the broad scale distribution of floral characteristics is now ripe for investigation (e.g., the Nov. 2000 issue of International Journal of Plant Sciences). To illustrate this potential, Barrett *et al.* (1996) supplemented earlier work (Schemske and Lande, 1985; Barrett and Husband, 1990) on mating system diversity and reanalysed the data in a phylogenetic context. The results of this enhanced study confirmed the earlier results and uncovered a novel influence on the evolution of plant mating systems. In the previous studies, that had not corrected for phylogeny, ecological variation between species had obscured this novel influence. However, much of this variation occurs at broad taxonomic levels and, so, variation is much higher between taxonomic groups than within them. The phylogenetic approach statistically removes this variation due to taxonomy and reveals the underlying evolutionary forces.

A recent investigation into the evolution of staminodes (Walker-Larsen and Harder, 2000) also employed the use of the angiosperm phylogeny. Furthermore, to investigate the evolution of this complex character in more detail, they included finer scale phylogenies of the Scrophulariales. This approach of investigating character evolution at multiple taxonomic scales revealed a great deal of information about the rate at which complex characters can be gained and lost.

This type of information is of fundamental importance to our understanding of adaptation as a dynamic process and can only be investigated through comparative methods. Further studies incorporating both broad and fine scale analyses of adaptation are certain to become commonplace as the techniques of molecular systematics continue to become more accessible.

Homology and homoplasy

Homology is a fundamental concept in the study of adaptation and, perhaps as a consequence, also a source of great disagreement (Donoghue, 1992). According to evolutionary theory organismal features can be similar for two reasons: common ancestry or convergent evolution. The former causes homology; the latter homoplasy. In fact Darwin (1859) used homology repeatedly as evidence for common ancestry in evolution. Without becoming too entangled in the debate, I favour Van Valen's (1982) definition that homology is a correspondence between two or more characteristics of organisms that is caused by continuity of information. In this case the continuity is caused by genealogy.

When using the comparative method to study adaptation, it is important to verify whether the structures being compared are in fact homologous. If, for example, your interest was in the role of petals in pollinator attraction, it is important to note that in some groups of plants (e.g., *Cornus*, *Dalechampia*) other parts of the plant are involved in attraction. The danger is that if you are not aware of this lack of homology in "petal-like" structures you may invoke strong selective pressures to explain any morphological differences when, in fact, they are expected to be different due to unique developmental and phylogenetic processes. Tracing the evolution of morphological characters onto the phylogenetic trees constructed from molecular systematics provides a potent mechanism for detecting homology. This is particularly true since molecular systematics does not use morphological characters in phylogeny construction. To continue the petal example, if one of your study species falls out on the phylogeny with a group of apetalous species, then the structures that you believe to be petals may in fact arise from a different structure. This occurrence of homoplasy (similarity that is not due to common ancestry, Sanderson and Hufford, 1996), is also an important biological phenomenon. The consistent appearance of similar solutions to the same challenge is strong evidence for evolution by natural selection. For example, the fact that plants evolve consistent responses to their pollinator's preferences (pollination syndromes Proctor *et al.*, 1996) demonstrates the potency

of natural selection and the availability of the requisite genetic variation to respond to these selective pressures. Both of these findings are important objectives in evolutionary biology. As with homology, tracing character evolution onto phylogenies reveals instances of convergent evolution which can then be focussed on for further study. The main point is that if homologous structures are similar to each other, common ancestry, rather than adaptation, is invoked as an explanation. Conversely, similarity between homoplasious structures suggests adaptation not common ancestry. Without phylogenies to make clear distinctions between homology and homoplasy, the proper expectation of similarity cannot be made and the role of adaptation in character evolution could be misinterpreted.

A final comment on the importance of homology in adaptation. Gould and Lewontin (1979) presented an influential critique of some of the methods used in the testing of adaptive hypotheses. One of their points was that adaptationists arbitrarily reduce organisms into traits, thereby destroying the context in which these traits operate. In response to this criticism, Larson and Losos (1996) have pointed out that homology of characters can be used to justify atomisation. If the trait changes in a coordinated and consistent way to specific selective pressures in different species, then studying that trait as a unique component of an organism is justified. The occurrence of homology through evolutionary time (as revealed by a phylogeny) removes any perceived arbitrariness to this procedure.

Molecular clocks

Another area of interest in the study of adaptation concerns the rate at which characters change over time. Darwin (1859) believed that evolutionary change was essentially gradual. Others have advocated a more punctuated tempo of evolution consisting of large periods of stasis followed by rapid change (Gould and Eldredge, 1977). The fossil record has been the main arbiter of this debate. However, fossils are well known for their scarcity and idiosyncratic creation (Darwin, 1859; Jablonski *et al.*, 1986). As such there has been a long standing hope for better techniques in reconstructing the age of evolutionary events. Early on in our understanding of protein structure and stability it appeared that the genetic composition of related proteins could be used as a molecular clock to reconstruct historical events (Zuckerkandl and Pauling, 1965; Thorpe, 1982). The basic idea was that although mutations occur at random locations in the genome, they occur at a regular frequency. If the average rate of mutation

could be measured, differences in genetic sequences could be used to infer the time that had elapsed since genes had diverged. Consider, for example, an enzymatic protein that accumulates neutral mutations at the rate of one base pair change every million years. Genetic sequences from copies of this enzyme for *Chamerion angustifolium* and C. *latifolium* might reveal that they differed by two base pairs. This would then imply that the species had separated from each other two million years ago (two ticks of the clock). Similarly, a third species, *Epilobium hirsutum*, with a five base pair difference would suggest that the *Epilobium* and *Chamerion* genera diverged five million years ago.

Unfortunately, the promise of this universal molecular clock has not materialised (see Hillis *et al.*, 1996, Chapter 12). Both empirical and theoretical investigations have demonstrated that the variance in age estimates provided by this approach is so large as to be useless. One of the fundamental problems is that the molecular clock idea assumes stable and consistent mutation rates across taxa. As it turns out, mutation rates are often unique to both the molecule of study and the taxon possessing the molecule. Consequently, there is no universal clock that can be used across all taxa.

However, rates of sequence divergence have been profitably used to investigate relative rates of divergence in closely related species. Despite the fact that mutation rates are variable at broad taxonomic levels, they are often sufficiently regular at fine taxonomic scales (such as the species and generic levels). This has become an important tool for investigating rates of adaptive change. For example, the genus *Aquilegia* shows extensive variation in floral morphology, pollination biology, and habitat type. Moreover, the species within the genus are largely interfertile, suggesting that they are genetically very similar. This genetic similarity implied a recent origin and striking adaptive divergence of the genus, which would make *Aquilegia* an excellent model group for the study of evolution (Clausen *et al.*, 1945). However, it was also possible that the wide variation exhibited by the genus was the result of an ancient divergence and that interfertility was not indicative of genetic similarity (Stebbins, 1950). To resolve this discrepancy, Hodges and Arnold (1994) sequenced sections of both the nuclear and chloroplast genome from 27 species of *Aquilegia*. Compared with related genera, the species of *Aquilegia* sampled had extremely low levels of genetic divergence (i.e., very few base pair changes between species), suggesting a recent and rapid period of evolutionary change. This rapid change was attributed to the evolution of the characteristic multiple nectar spurs of the genus. This key innovation allowed for specialisation with specific pollinators and led to concordant changes in

floral morphology and ecological habitat. In the absence of the molecular techniques that allow for such an investigation, the time scale over which evolution can operate would be largely unknown and would represent a serious gap in our understanding of adaptation.

Statistical independence

This section describes an important methodological issue that pertains to any comparative study of adaptation. Consider the hypothesis that nectar volume influences the frequency of self-fertilisation through its effect on pollinator visitation patterns (e.g., Hodges, 1995). The scenario is that high nectar volumes induce pollinators to linger at a flower. This lingering causes an increase in pollen transfer from anther to stigma within the flower, causing an increased frequency of self-fertilisation. Figure 2.3 presents a possible scatter plot of selfing rates and nectar volumes for 19 species. The relationship shown demonstrates a positive correlation between nectar volume and selfing rates, in accordance with the hypothesis being tested. A linear regression based on these data would no doubt yield a highly significant R^2 (based on 19-2=17 degrees of freedom). However, the story changes drastically when the taxonomic distribution of the species is included (Figure 2.4). The statistical assumption of independent data points is severely violated due to the shared history of the species within each genus. Strictly interpreted there are only two independent data points; one for *Aquilegia* and one for *Epilobium*. This presents two serious problems. The first is a severe reduction in sample size (from 19 to 2). Consequently much more work is required to generate enough data to rigorously test the hypothesis under study. The second difficulty is the miscalculation of degrees of freedom in the statistical test. Since there are only two data points, the appropriate test here would be a t-test which would yield only 1 degree of freedom (2-1). Other statistical issues aside (e.g., replication), such an error leads to an increased chance of a Type I error (rejecting a true null hypothesis). Consequently, not taking phylogeny into account may result in accepting the false conclusion of an association between nectar volume and selfing.

This difficulty has been recognised by biologists for some time. However it was not until Felsenstein's (1985) influential paper that the issue was taken more seriously. Perhaps the main reason for this influence was that Felsenstein presented a method for identifying independent evolutionary events that could be used as the basis of statistical tests. This method uses a phylogeny to construct multiple independent contrasts that can be used to evaluate an adaptive

hypothesis. In addition to its statistical rigor, by generating a hierarchy of comparisons high degrees of freedom can be maintained for powerful tests (see Harvey and Pagel, 1991). With proper phylogenies for *Aquilegia* and *Epilobium*, using the comparative method of Felsenstein (1985) would actually give 18 degrees of freedom (the number of species -1), making for a more powerful statistical test and a greater chance of uncovering the true relationship between nectar volume and selfing rates.

This discussion has been couched in statistical terms, however the statistical issues are generated by the fact that evolution is an historical phenomenon. The data of Figures 2.3 and 2.4 may cluster due to common descent, rather than adaptive reasons. Of course this too is an hypothesis that should be rigorously tested rather than assumed (Westoby *et al.*, 1995). The important point here is that without proper phylogenies such issues cannot be investigated.

Caveats

Although this renaissance of the comparative method is a welcome and valuable development, it is not a panacea for all studies of adaptation. The ideal approach to studying adaptation is found in the cooperative use of both the comparative and experimental methods. Comparisons at broad taxonomic levels are excellent for investigating general trends in biology and for suggesting testable hypotheses. They are not suitable for fine detail studies of adaptation. This is recognised in the methodology suggested by Baum and Larson (1991) for the study of character evolution. They advocate a pluralistic approach, where experiments provide functional evidence for adaptations while comparisons elucidate the historical aspects of adaptation. Perhaps the major difficulty with molecular systematics is the often poor resolution of the phylogenies produced. If the majority of character variation occurs below the resolution of a phylogeny, the comparative method may not be very illuminating. However, as techniques continue to improve and are applied to more groups, phylogenies will increase in both precision and efficacy. Furthermore, theoretical analyses suggest that, even with poorly resolved phylogenies, incorporating phylogenetic information into comparative analyses provides more accurate results when compared with studies that ignore relationships among species Purvis *et al.* (1994). Type I errors (rejecting a true null hypothesis) were found to be very robust to poorly resolved phylogenies. However, type II errors (accepting a false null hypothesis) were adversely affected. This suggests that comparative methods yielding negative results (i.e., $P > 0.05$) should be

interpreted cautiously. However, given biologists' abhorrence of negative results, this fact may also provide incentive to enhance the resolution of angiosperm phylogenies.

Conclusions

In the study of adaptation, each of the difficulties described above are resolved with phylogenetic reconstruction ("tree thinking" Maddison and Maddison, 1992, Chapter 3). Molecular systematics has made important contributions to these phylogenies by providing efficient and convenient techniques for their construction. Accordingly, with the comparative method resurrected and well equipped, it can be coupled with experimental approaches to yield important insights into the evolutionary process.

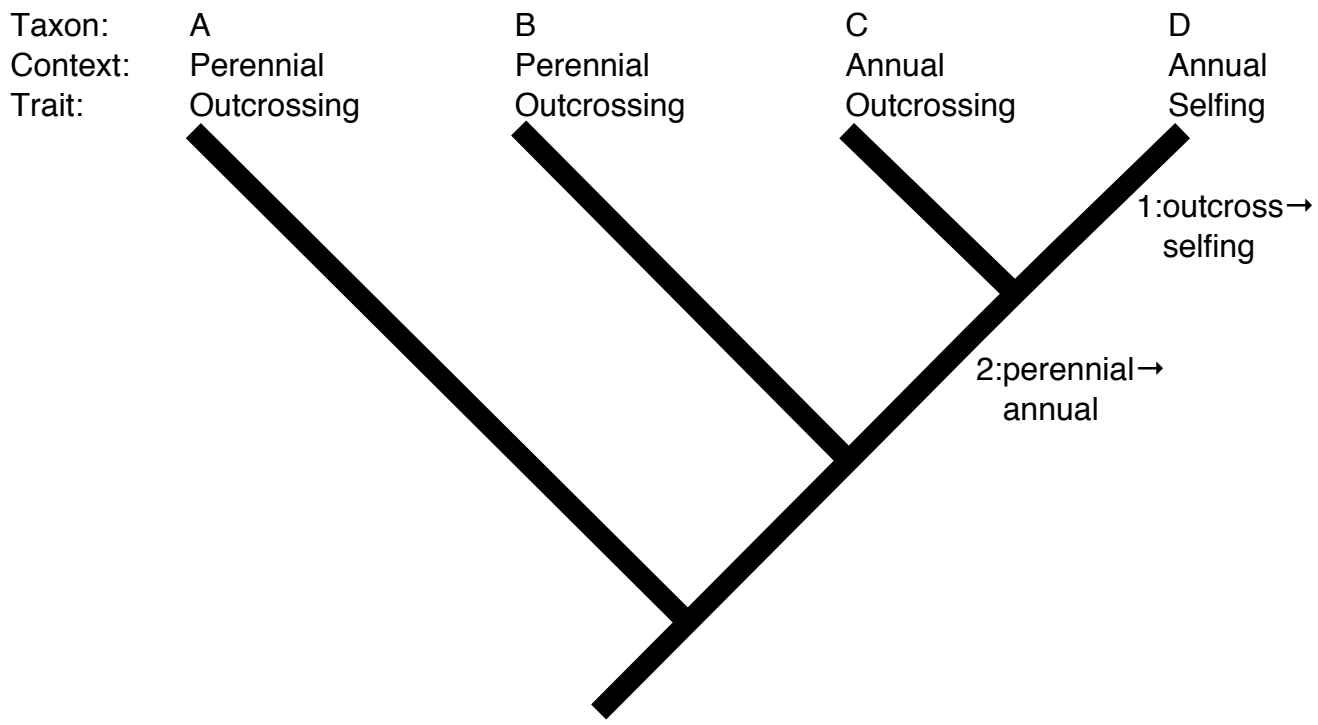

Figure 2: The phylogenetic tree for four species of angiosperms with their associated life-history and mating-system characteristics mapped onto the tree. This phylogeny supports the hypothesis that selfing evolves in response to the evolution of the annual habit.

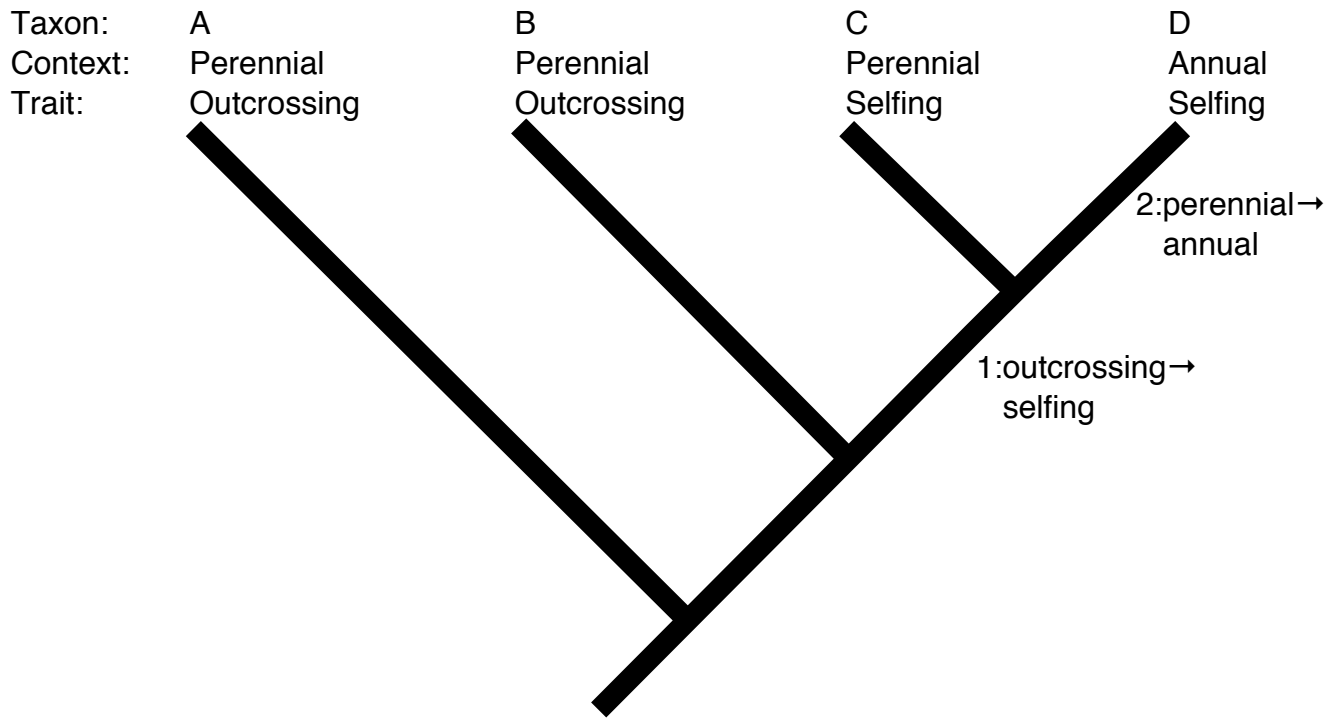

Figure 3: As in Figure 2.1, except that in this case the hypothesis is not supported. Notice that the evoution of selfing precedes the annual habit.

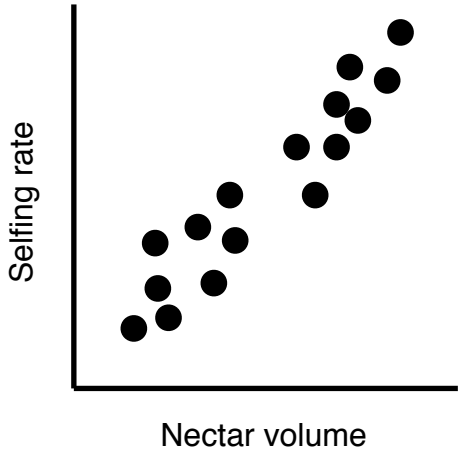

Figure 4: A distribution of nectar volumes and selfing rates for 19 species of angiosperms. This pattern suggests an association between the two characters.

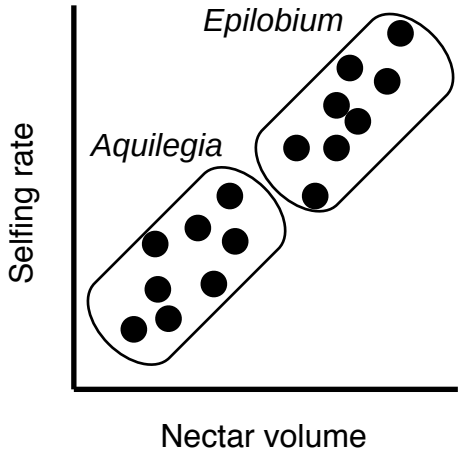

Figure 5: The same data as in Figure 2.3, with the addition of phylogenetic information. The clustering of points now suggests a strong effect of taxonomic membership on the association between nectar volume and selfing rates. This substantially weakens the evidence for the hypothesis supported by Figure 2.3.

Constraints and adaptation

> Evolution is henceforth the magic word by which we shall solve all the riddles that surround us.
> Ernst Haeckel

Constraints generate diversity. This may seem odd given that a constraint, in the common usage of the term, is a limitation on motion or action. One might expect that a limitation could only restrict the number of possible biological types. However, in the absence of compromises, impossibilities, and pragmatic choices generated by these limitations, life would consist of a single, maximally adapted life form that dominated all habitats. Evidently this is not the case; biology is characterised by its enormous diversity of form and function. Since biologists are fascinated with diversity, the identification and potency of constraints enjoys a long and rich history. Unfortunately the use of constraints suffers from two abuses. The first is the application of the concept itself and the second is the profusion of constraint terminology. I will illustrate the first abuse with a discussion of optimality models. This will be followed by a review and synthesis of constraints with illustrations from plant sexual systems. In the end, once constraints are rigorously implemented and incorporated into a coherent framework, they retain their position as a fundamental aspect of evolution.

Optimality and constraints

Optimality models are an important tool in the study of adaptations (Parker and Maynard Smith, 1990; Seger and Stubblefield, 1996). The basic optimisation approach is characterised by two assumptions: that fitness is maximised and that there are inherent trade-offs between components of fitness. Seed production represents a classic example of the optimality

approach (e.g., Smith and Fretwell, 1974). Given that a plant has a fixed portion of resources available for seed production, the extreme allocations would be one massive seed or many small seeds. The large seed may have enhanced survivorship due to its store of resources, but many small seeds potentially result in greater fecundity for the parent plant. Optimality models can be used to predict where a plant's reproductive strategy should be along this continuum.

In practice, optimisation models consist of three steps (Seger and Stubblefield, 1996):

1. Construct a model (generally mathematical) that represents the situation under study including all fitness trade-offs

2. Calculate from the model the set of character states that maximises fitness

3. Test the predicted value with relevant empirical data

Optimality models themselves are not germane to this discussion, so this caricature of the approach should be sufficient. A common criticism of optimality approaches revolves around the third step (van der Steen, 2000). If the predicted value does not meet the empirical data, one strategy is to invoke a constraint to explain the discrepancy. This rescues the model and confirms that the trait is indeed optimally fit. The criticism is that these constraints are often *ad hoc* and untested (Perrin and Travis, 1992). If constraints are to be a useful concept in evolutionary biology, they should be treated as hypotheses to be tested rather than band-aids for a clever theoretical model with no empirical support.

Taxonomy of Constraints

An additional consequence of this implementation of constraint at the first sign of non-adaptation is that the evolutionary literature is replete with constraint terminology. The situation was so dire that Antonovics and van Tienderen (1991) called for a temporary moratorium on constraint usage (see also Perrin and Travis, 1992; van Tienderen and Antonovics, 1994). However, on further examination these constraints collapse into general types (e.g., Gould, 1989; Arnold, 1992). Rather than generate constraints on a case by case basis, the ideal solution would contain a set of general types of constraint. The use of these restricted definitions would greatly simplify the search for a common underlying framework of adaptative processes in all species. This is essentially an inductive process (moving from specifics to generalities)

and mirrors the successful incorporation of natural history into evolutionary biology with its concomitant enhanced potency. Based on the literature cited above, there appear to be four candidates for general constraint: genetic, phylogenetic, developmental, and formal. Before describing these general constraints, it is important to point out that fitness trade-offs should not be considered as constraints on adaptation. These trade-offs are often described as selective constraints. However, selection should not be viewed as a constraint. Rather, selection represents an evolutionary force (Gould, 1989). Consequently, I have not included it as a general type of constraint.

Genetic constraints

Genetic constraints can perhaps be best illustrated by the breeder's equation:

$$R = h^2 S$$

Where a population's response to selection (R) is a product of the selection coefficient (S) and the heritability (h) of the trait under selection (Falconer and Mackay, 1996). Heritability is essentially a measure of the resemblance of offspring to their parents that is attributable to genetics. In the absence of genetic variation, heritability is zero and there will be no response to selection regardless of its strength.

The breeder's equation represents the simple case of a trait controlled by a single gene in isolation from other genes. More complex and biologically realistic models have been developed that allow for multigenic traits and character correlations (Lande, 1979; Lande and Arnold, 1983). In these more sophisticated models, heritability is replaced by a genetic variance-covariance matrix (the $\mathbf{G}$ matrix) and selection is represented by a vector of selection gradients (β). The change in trait means (z) due to selection then becomes a matrix product:

$$\mathbf{\Delta \hat{z}} = \mathbf{G}\beta$$

As with the breeder's equation, the response to selection is constrained by genetic variance. However, in this case it is also constrained by the genetic covariance between traits. The coupling of variance and covariance represents a potent constraint on adaptation. In fact, as I will argue below, genetic constraints represent the proximate cause of all constraints on adaptation.

In addition, there is considerable debate over the stability of the **G** matrix. It may be possible for selection to act on genetic constraints by modifying the connections between genes. Fisher (1930) argued that constant selection will reduce genetic variance and eventually eliminate all heritable variation (his fundamental theorem of natural selection). This implies that long-term selection will always be constrained by a lack of heritability. However, Lande (1980) suggested that a trait controlled by many loci will always have a significant pool of mutation to work with. This is an important issue since a labile **G** matrix would erode genetic constraints, while the converse would enhance them. Empirical investigations suggest that **G** is stable but flexible enough for selection to modify (e.g., Arnold, 1992; Schluter, 1996). In other words, given sufficient time selection can modify genetic constraints.

The strength of genetic constraints can be examined with artificial selection experiments. Stanton and Young (1994) performed such an experiment on the ratio between petal size and pollen production in *Raphanus sativus*. Cross-trait covariances between parents and offspring suggested a correlation of 0.64-0.84 between these two characters which suggested a strong genetic correlation. However, despite this strong relationship, after just two generations of selection petal:pollen ratios were changed by $\sim$40% with no appreciable loss in genetic variation. This demonstrates the importance of rigorously investigating putative constraints. Without this experiment, such a high genetic correlation would have been considered a strong constraint on adaptation. Moreover, any concerted response to selection of these two traits would have been attributed to constraints when in fact adaptation was promoting their response. Only rigorous testing of constraints as hypotheses can prevent such a misinterpretation.

Phylogenetic constraints

One of Darwin's (1859) important insights into the evolutionary process was that life is connected through a series of ancestor-descendant relationships (phylogeny). Consequently, evolutionary choices made by ancestors constrain the responses available to their descendants. Furthermore, the ramifications of these locked-in choices are exacerbated by changing environmental conditions and selective pressures that may favour trait values that are no longer available due to past selective pressures. It is important to point out that these phylogenetic constraints only apply to monophyletic taxa. In polyphyletic taxa, unrecognised convergent evolution could give the impression of phylogenetic constraints when, in fact, unconstrained

adaptation has caused trait values to converge.

Kochmer and Handel (1986) investigated three explicit hypotheses for the evolution of flowering phenology in animal-pollinated angiosperms. First, that flowering time is selected to reduce competition for pollinators (unconstrained adaptation). Second, that flowering time is a result of taxonomic membership (phylogenetic constraint). Third, that flowering time is a consequence of life form (adaptive trade-offs). A comparative analysis of almost 4,000 angiosperm species found that family membership explained the majority of the variance in flowering time. This suggested that phylogeny has played a strong role as a constraint on the evolution of phenology.

Developmental constraints

It seems to me that many of the developmental constraints discussed in the literature (e.g., pleiotropy, epistasis, canalisation) would be better placed within genetic constraints. Nonetheless organisms must maintain internal coherence throughout the ontogenetic process (Schlichting and Pigliucci, 1998) and I believe that this represents a developmental constraint. It is worth noting that until recently development had been treated as a black box into which genotypes entered and phenotypes emerged (Schlichting and Pigliucci, 1998). As a consequence the role that developmental constraints are thought to play in evolution is certain to change drastically as our knowledge of the ontogenetic process increases. They are included here for completeness and with the expectation that they do represent a potent constraint.

An example of developmental constraints can be found in the ontogeny of symmetry in flowers (Tucker, 1999). In most radially symmetric flowers (e.g., *Silene*, *Magnolia*) once a flower is initiated its symmetry cannot be changed during subsequent development. Consequently, selection for changes in symmetry at later developmental stages are constrained by this ontogenetic process.

Formal constraints

Formal constraints are also called mechanical or physical constraints. These are constraints on adaptation due to physical laws and result from the interactions between biology

and the physical world. Unlike the other constraints listed, I consider formal constraints to be immutable. For example, the conservation of energy and gravitation are not responsive to selective pressures. Not surprisingly, formal constraints are often investigated with the language and techniques of engineers, since their main concern is the effects of physical laws and forces on structures.

Karl Niklas is particularly adept at investigating formal constraints. Selecting one example from his work, Niklas (1993) investigated the relationship between plant reproductive biomass and stem diameter. Presumably, as reproductive biomass increases (i.e., flowers grow larger) the diameter of the stem attached to the reproductive structure must also increase to support the additional weight. An examination of representative mosses, pteridophytes, gymnosperms, and angiosperms demonstrated that this was the case and that equations used in structural design could be profitably applied to living organisms.

Synthesis

With constraint terminology distilled and described, a framework can be constructed that explicitly states the relationships between the types of constraint. This framework is useful for two principle reasons. First, it circumscribes the domain of each constraint and obviates the need to create new terminology. Second, it unifies constraints into a common conceptual paradigm that suggests approaches to investigating the nature of constraints on adaptation.

Figure 3.1 illustrates adaptation in the Panglossian world of no constraints. In this scenario selection acts on phenotypes in a given environment to produce an optimally fit organism. This selective pressure includes all trade-offs in the components of fitness for the population under study. As such it is equivalent to the basic optimisation approach to adaptive hypothesis testing. Additionally, since this is an unconstrained model it is assumed that the phenotype maps directly onto the genotype. Constraints can be visualised as a deflection from this optimal phenotype predicted from the optimality model (Figure 3.2). At the centre of this model is the **G** matrix which represents genetic constraints. The proximate cause of the deflection caused by constraints is either a lack of genetic variation or an antagonistic covariance between loci controlling a trait value. The lability of **G** is represented by the circular arrow which allows for selective changes in genetic architecture. Conspicuously absent from this model are mutation, genetic drift, and other stochastic processes of evolution. They could be included as modifiers

of **G** in a more complex model. However, in the interests of focusing on constraints, I have not explicitly included them here.

I consider the ultimate causes of constraints to be the effects of phylogeny, development, and physical laws on **G**. This represents an inversion of Arnold's (1992, pg. S94) views where genetic constraints are the ultimate cause while developmental and functional constraints are the underlying proximate causes. I view genetic constraints as the answer to how adaptation is constrained and phylogenetic, developmental, and functional constraints describe why there are genetic constraints. I believe that this produces a more integrated and simplified view of constraint that I will describe below.

The legacy of history leaves populations with a particular level of genetic variation and pattern of genetic architecture (e.g., Harvey and Pagel, 1991, Chapter 2). This genetic legacy is not idiosyncratic; it represents a long series of selective pressures and compromises. With each generation these decisions are made and locked into **G**. Over time this builds up into a phylogenetic inertia (represented by the phylogenetic constraint box of Figure 3.2) which modifies the value of **G**. However, the restrictions that phylogeny places on selection can be modified over time by selection on the genetic architecture of populations and by input from mutation and migration. If this were not the case evolution would grind to a halt from the weight of its phylogenetic load.

The evolution of multicellularity resulted in a tremendous increase in life's diversity (Futuyma, 1998, Chapter 7). It may also represent the origin of developmental constraints. Once organisms needed to coordinate the growth and function of their constituent cells, epigenetic relationships arose to ensure the developmental coherence of the organism. This requirement of coherence restricts genetic architecture (perhaps through epistasis and pleiotropy). Thus development constrains **G** resulting in genetic constraints that deflect selection from an ideal phenotype. As with phylogenetic constraints, selection can act on **G** to modify developmental constraints. However, the degree to which ontogeny can be modified by selection is currently the subject of much debate (Schlichting and Pigliucci, 1998).

Of course organisms live in the physical world and are constrained by natural laws. These laws can place strong constraints on adaptation and, unlike the other two constraints, are not malleable by selection (represented by the thickness of the arrow in Figure 3.2). Formal constraints operate by essentially prohibiting the subset of all possible **G** matrices that violate natural law. Nonetheless, "life has found many surprising ways of side-stepping [formal

constraints]" (Niklas, 1997, pg. 249).

The model of constraints presented here suggests a research method for the investigation of adaptation that explicitly treats constraints as testable hypotheses. Figure 3.1 represents the null model to be tested (Antonovics and van Tienderen, 1991). Following the optimality approach described above, if the theoretical prediction from Figure 3.1 did not meet the empirical data the model in Figure 3.2 could be invoked. Since constraints on adaptation revolve around genetic constraints, an investigation of $\mathbf{G}$ is the appropriate place to start. Heritability studies or artificial selection experiments could provide convenient tests of genetic constraints. If high heritability or responses to artificial selection suggest that a putative genetic constraint does not exist then there is no need to move to the next level in the hierarchy of constraints (i.e. phylogenetic, formal, developmental). Instead the assumptions of the optimality model should be revised to incorporate new trade-offs or assumptions. However if a genetic constraint is found an investigation of the next level of constraints would be warranted. The techniques used to investigate the nature of these constraints were briefly discussed above. Phylogenetic constraints can be examined by partitioning character variation into population, species, and higher taxonomic level components (e.g., Armbruster, 1988, 1991) or other techniques of comparative analysis (e.g., Harvey and Pagel, 1991). Matching organismal design to engineering principles can elucidate formal constraints (e.g., Niklas, 1993). Finally, investigating developmental constraints may be amenable to the emerging developmental reaction-norm perspective (Schlichting and Pigliucci, 1998).

It is important to point out that these constraints do not act in isolation of each other. Genetic constraints may be a consequence of a combination of all three higher level constraints. In other words, adaptation may be deflected by an interaction between constraints. This is similar to the points raised by Westoby *et al.* (1995) for disentangling the effects of ecology and phylogeny. The resolution of this issue is beyond the scope of this discussion. However, the techniques of path analysis may be useful here (e.g., Queller, 1997). Path coefficients could be estimated for each arrow in Figure 3.2 and the magnitude of these coefficients would represent the strength of each constraint on the adaptation under study. The primary difficulty is that calculating interactions between constraints requires replication at each level of the study (as in ANOVA designs, see Neter *et al.* 1996 Part IV) which may not be possible for all adaptive studies.

Constraints in plant sexual systems

Constraints that are universal for and unique to the angiosperms are difficult to find. Certainly at lower levels (i.e., populations, species, genera) there are characteristic constraints that mold adaptive responses. However, are there any universal constraints for the approximately 250,000 species of angiosperms?

The evolution of the characteristic enclosed carpel is thought to have promoted the success of angiosperms (Faegri and van der Pijl, 1979; Proctor *et al.*, 1996). Two major benefits of this evolutionary novelty are protection of the ovules from insects and the subsequent evolution of the stigma and style (allowing for self-incompatibility, mate choice, etc.). However, since the evolution of the flower and enclosed carpel is shared by all angiosperms it is also a possible source of a universal constraint. Indeed this possibility was investigated by Primack (1987), although he did not describe it as such. Through a combination of anatomical, genetic, physiological, and empirical evidence Primack demonstrated strong continuity from flowers to fruits to seeds. Consequently, due to their shared developmental pathways and phylogenetic history, selection cannot modify any of these structures in isolation (i.e. both developmental and phylogenetic constraints are operating to produce a genetic constraint). For example, evolutionary changes in flower size were found to be correlated with changes in fruit size. This constraint then percolates throughout the rest of pollination biology. The evolution of small seeds to enhance dispersal would result in a decrease in flower size as well. If later selective pressure favour large flowers to increase pollinator attraction, the early evolution of seed size would impinge on the plant population's ability to respond. This tension between seed and flower sizes causes a deflection in the path of adaptation. Due to our understanding of the underlying genetic architecture of this tension, we can characterise it as a constraint on adaptation. In addition, due to our phylogenetic and developmental knowledge, we can further characterise the constraint as a combination of phylogeny and development.

Conclusions

Constraints on the course of adaptation represent potent forces in evolutionary biology. Unfortunately the use of constraints in the literature suffered from lax implementation and a profusion of terminology that obscured their meaning. Evolutionary biologists have subse-

quently recognised these problems and largely corrected them. Constraints are now tested as hypotheses, rather than simply implemented *ad hoc*. In addition, the recognition of underlying commonalities to the types of constraints has led to a distillation to a handful of general types. Hopefully the framework presented here is a useful one that both simplifies the issue and provides an outline of a research method. Given the enormous literature devoted to this topic, the controversy is certain to remain in the short term. However, a consensus appears to be forming that should allow for a focussed investigation of the role of constraints in evolution.

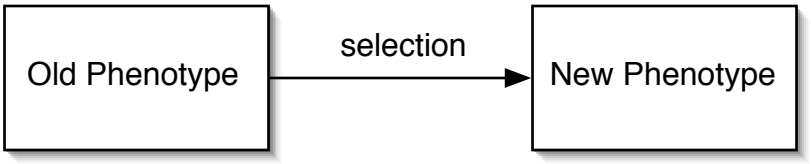

Figure 6: Adaptation in a constraint-free world. Phenotypes are completely responsive to selection and the optimal phenotype for a given environment evolves.

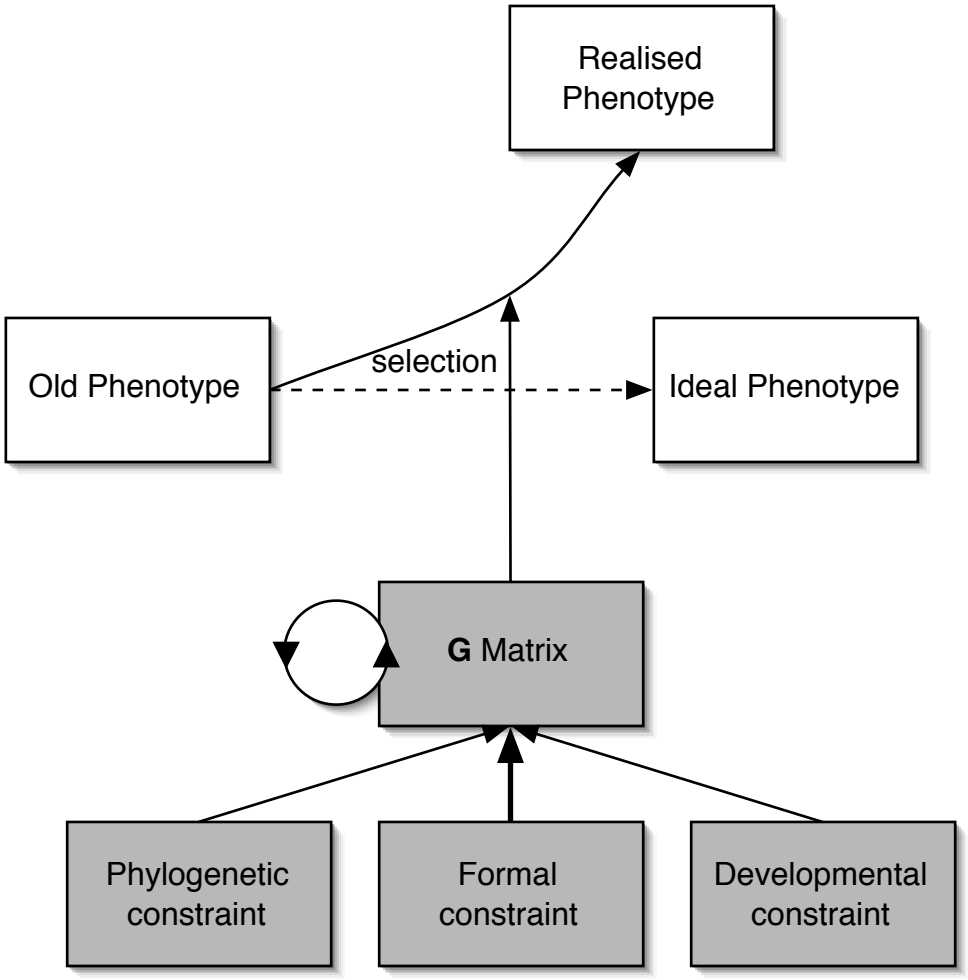

Figure 7: In contrast to Figure 3.1, the natural world contains constraints that deflect selection from an ideal to a realised phenotype. See text for details.

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