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Floral mimicry: a fascinating yet poorly understood phenomenon

Bitty A. Roy and Alex Widmer

Flowers of different species that resemble each other are not necessarily mimics. For mimicry to be occurring, the similarity must be adaptive. Unfortunately, no case of floral mimicry has ever been fully verified and it is important that we move beyond these perceived similarities to testing whether they are truly adaptive. Here we explain the differences between Batesian and Müllerian floral mimicry, illustrate what should be done to test mimicry hypotheses, and discuss how interspecific pollen transfer influences the evolution of mimicry.

The concepts of Batesian and Müllerian mimicry have been developed by zoologists and are commonly associated with protective mimicry in animal systems[1,2]. However, these concepts also apply to plant systems because the same evolutionary processes that form them (negative and positive frequency-dependent selection) occur whether an animal is being warned away (protective mimicry) or invited in (floral mimicry) (Fig. 1). In animal Batesian mimicry, selection favors resemblance of a palatable mimic to an unpalatable model. Similarly, in Batesian floral mimicry, selection favors resemblance of a non-rewarding mimic to a rewarding model[3,4]. In animal Müllerian mimicry, selection favors convergence on a single, 'aposematic', warning pattern as a defense against predators, such as the yellow and black striped pattern of bees, wasps and hornets. Similarly, in floral Müllerian mimicry, selection favors similar floral appearance among rewarding plants for the sake of attracting pollinators. The view that some floral mimicry systems fall within the concept of Batesian mimicry is now well established[3–5] although experimental tests remain few. Floral Müllerian mimicry is both less commonly accepted and less studied.

For floral mimicry to be established as occurring between two or more similar species, they must:

- Have strongly overlapping distributions, and must have done so long enough for coevolution to have occurred.
- Require pollinators for seed set.
- Overlap substantially in flowering phenology.
- Share the same pollinator species and the same individual pollinators must move freely between the species.
- The similarity must be important for fitness[6–8].

The majority of floral mimicry studies establish the first four points, but either neglect or incompletely address the last point – the critical question of whether the similarity is actually adaptive. Before we suggest the tests necessary to assess the fitness consequences of similarity, we would first like to further describe the basic kinds of floral mimicry, because the type of mimicry influences the kinds of tests performed.

There are two basic types of floral mimicry, Batesian and Müllerian, which are governed by different selection regimes (Fig. 1). In Batesian floral mimicry, the mimic produces no nectar reward, whereas the model does (Fig. 2). Hence the mimic's chances of visitation should be increased through its similarity to a nectar-producing model. Further, because the Batesian mimics do not have nectar, the more frequent they are in the population, the lower their pollination success becomes because pollinators can learn to avoid flowers that look a certain way, and indeed, both mimic and model might be avoided[9]. Thus, new Batesian mimic phenotypes that mimic a different model will enjoy a pollination advantage and this type of negative-frequency-dependent selection should select for increased diversity of model–mimic pairs (Fig. 1).

In Müllerian floral mimicry, two or more rewarding flower species gain a collective advantage as a result of convergence on a 'common advertising display'[4,7,10–15]. The similarity of Müllerian mimics increases the 'perceived' density of rewarding flowers and, thus, might increase the probability of pollination (Fig. 3). When pollinator visitation is positively density-dependent, greater similarity among flower species implies higher pollination success. Thus, Müllerian mimics are undergoing positive frequency-dependent selection (Fig. 1), and are all converging on a similar phenotype. In spite of selective pressure towards similarity, variation in Müllerian mimics probably exists because pollinators

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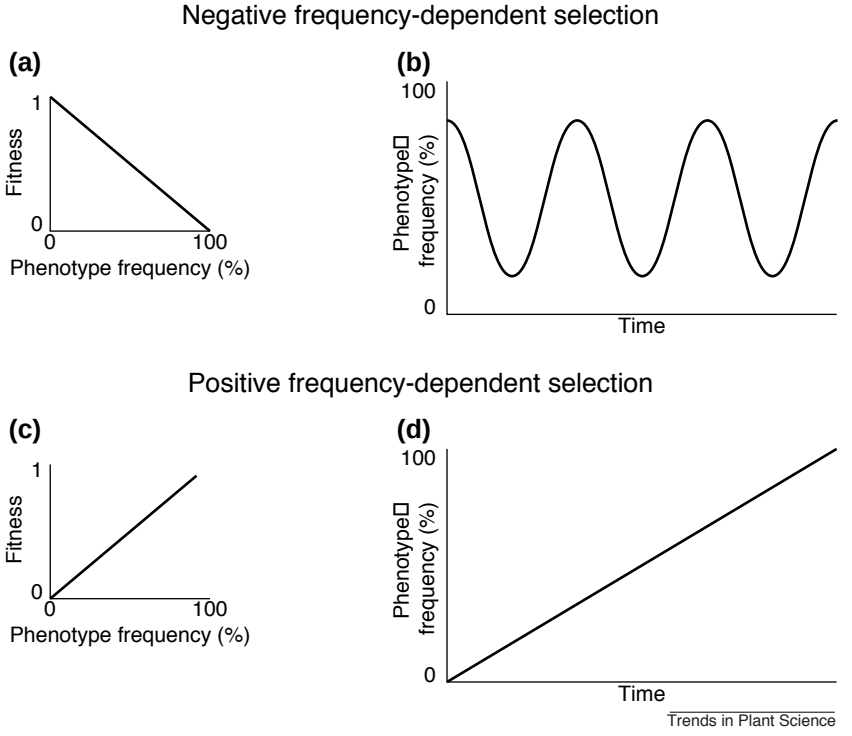

Fig. 1. Frequency-dependent selection. (a) Batesian mimicry is molded by negative frequency-dependent selection. The rarer a phenotype, the higher its fitness. As a rare phenotype becomes more common, its fitness will decline, leading to a decrease in frequency. (b) Thus, in negative frequency-dependent selection, the frequency of a phenotype will vary over time. (c) Müllerian mimicry is molded by positive frequency-dependent selection. The more common a phenotype is, the higher its fitness. This means that over time, the more common phenotype is favored by selection and eventually becomes fixed in the population (d). Therefore, little or no variation is expected in Müllerian mimics. (b) and (d) represent 'idealized' states. It is not known whether negative frequency-dependent selection by pollinators will lead to limit cycles of the exact form depicted, and positive frequency-dependent selection is unlikely to be consistent enough to lead to a straight-line function.

vary across the range of a species, and might change from one part of the season to another[16–18].

Several differences between the two basic types of floral mimicry should now be clear. In Batesian mimicry, only the model is rewarding, whereas in Müllerian mimicry, all the species present pollinators with rewards. In Batesian mimicry, the model does not gain from the interaction, whereas in Müllerian mimicry, all the species gain an advantage as a result of their similarity. In Batesian mimicry, there is an obvious model on which the mimic is based, whereas in Müllerian mimicry, there is not always an obvious model or mimic – all the taxa involved are converging towards one another. However, the commonest species (or phenotype) should have the highest fitness, and is thus the model for all of the rest. A species that starts out as a Müllerian mimic can itself become the model if it becomes more abundant than the original model[19].

To test whether resemblance is adaptive in a Batesian system, one needs to establish that the mimic receives more visits and has higher fitness when the rewarding model is present than when it is absent[6,20]. Both patch density and composition probably influence pollinator behavior. When the patch is dense, or when the mimic is common, it is predicted that the pollinators will encounter unrewarding mimetic flowers more often, and fitness will thus decrease[6,20]. There are only a few studies of Batesian mimicry that have measured fitness in the manner described here (Fig. 2).

To test whether resemblance is adaptive in a Müllerian system, one should test whether individuals of the rarer species (which are thus termed the mimics) have higher fitness when they are most similar to the more common species (the model). However, these tests rely on there being phenotypic variation in the mimic, and there might not be variation if positive frequency-dependent selection has run its course and, thus, all of the mimics have the same characteristics. For example, it is impossible to test the Müllerian mimicry hypothesis that bees, wasps and hornets gain a collective advantage from their stripes warning away predators when all of the individuals have stripes.

There are two alternative methods for testing floral Müllerian mimicry hypotheses when there is little or no phenotypic variation in the putative mimics. First, one could create artificial phenotypes that lack the features associated with the mimicry, and test whether these individuals have lower fitness than those with greater similarity to the model. Second, one could determine whether the putative mimics have the same or even higher fitness when they co-occur in a patch as they do in separate, same-density patches of the individual species. In other words, do insects treat all the members of a Müllerian mimic ring as if they were the same, choosing when to forage based on the number or density of similar shaped, colored or scented flowers, rather than on the specific species composition of the patch? Some preference by pollinators might occur, but, on average, patch density, not patch composition should determine the fitness of flowers of similar appearance.

Interspecific pollen transfer and evolution of mimicry

There is a limit to how similar co-flowering, sympatric species can be to each other, because individuals need to be able to mate with the proper species. If two flower species look too much alike, visitors might transfer their pollen to the wrong species, a phenomenon called improper pollen transfer. Improper pollen transfer, which is a form of competition, will tend to select for differences among species[21–23]. A plant's success will be enhanced if a pollinator transfers its pollen directly to another of the same species. Thus, improper pollen transfer could reduce selection for mimicry unless it is ameliorated in some way.

Because there are examples of floral mimicry, we can look at those species to learn how improper pollen transfer can be decreased. Orchids, the group with the most mimetic species (up to a third of the known orchids, or ~10 000 species according to some estimates[24,25]), package their pollen in saddlebag-like structures called pollinia. It is suggested that pollinia are less likely to be improperly transferred than pollen because pollinia tend to be like keys, fitting only into flowers with the proper shape[26]. However, in spite of pollinia, hybridization is thought to be common in orchids[24]. Another trait that might be important for co-flowering species is flower longevity[27,28]. As long as the likelihood of hybridization is low, and the presence of foreign pollen on a stigma is not in itself harmful, mimetic flowers that can wait until the proper pollen arrives will have an advantage. The evolution of the *Costus allenii–C. laevis* mimicry system (Fig. 3) might have been favored by a combination of *Costus* flower longevity and strong barriers against hybridization[7]. The pseudoflowers produced by flower-mimic fungi are also long-lived, producing nectar for

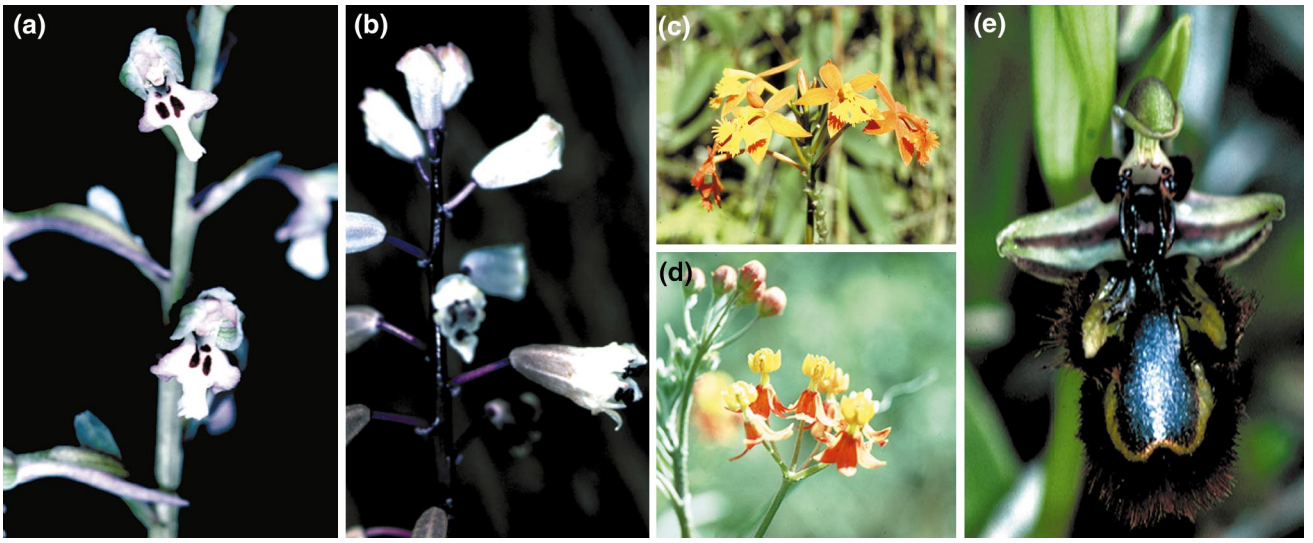

Fig. 2. Possible examples of Batesian floral mimicry. (a) Evidence for the existence of Batesian floral mimicry in orchids was found for the orchids *Orchis israelitica*, which mimics (b) the lily *Bellevalia flexuosa*[20], and *Disa ferruginea* (not shown), which mimics either *Tritoniopsis triticea* (Iridaceae) or *Kniphofia uvaria* (Asphodelaceae, not shown)[3]. In both of these systems most of the conditions for mimicry have been established. The putative mimics overlap in distribution and flowering time with the model, they require visitation for full seed set, and they share pollinators with the model. However, evidence for the condition that similarity must increase fitness is only circumstantial. Higher reproductive success (i.e. pollination and pollinia removal[3] as well as capsule production[3,20]) in the presence of the model, compared with situations where the model is absent, suggests that the resemblance of the mimic to the model does indeed account for the increased reproductive success. However, a similar result might also be observed in cases where non-rewarding, non-mimic flowers grow together with rewarding flowers, such as in *Orchis caspia*[52]. Therefore, to prove that *O. israelitica* or *D. ferruginea* are indeed Batesian mimics, experimental evidence is needed to demonstrate that mimics with greater similarity to the models have higher fitness than those that are less similar. Photographs (a) and (b) by Alex Widmer. (c) *Epidendrum radicans* (Orchidaceae), a non-rewarding orchid, was thought to be a Batesian mimic of the rewarding neotropical weeds (d) *Asclepias curassavica* (Asclepiadaceae) and *Lantana camara* (Verbenaceae, not shown) based on their similar floral appearance and pollinator sharing. However, contrary to expectations, a detailed study[6] revealed that flowers of *Epidendrum* growing interspersed with its presumed models were not visited more often than when growing alone, suggesting that the floral similarity between the presumed mimic and its models is not adaptive. Possible explanations might be that (1) the importance of floral mimicry changes with time and was not picked up during the restricted time-frame of the study; (2) that co-evolution has not yet occurred because the two rewarding species are not native to Central America; or (3) that the floral similarity is indeed not adaptive and these species are not mimics[6]. Photographs (c) and (d) by Paulette Bierzychudek. (e) Flowers of the orchid genus *Ophrys* represent a special type of floral Batesian mimicry. The flowers of *Ophrys* mimic female Hymenopterans and are pollinated by the deceived males when they attempt to copulate with the labellum (pseudocopulation)[53]. This type of pollination is known as pollination by sexual deceit. The interaction is presumed to be highly specific because *Ophrys* flowers need to provide the key stimuli to elicit male mating behavior. These stimuli involve optical cues, but also tactile and olfactory stimuli. Floral volatiles imitate the species-specific female pheromones of the Hymenoptera and are thought to be crucial because they directly act on the innate behavior of the pollinators (Ref. 5 and references therein). Pollination in *Ophrys* differs in one important aspect from other examples of floral Batesian mimicry: the phenologies of the mimics and the model only partly overlap and pollination success might drop dramatically after the emergence of female Hymenoptera. *Ophrys* flowers are most attractive during the short time window after the emergence of males and before the emergence of female Hymenoptera. Photograph (e) by Alex Widmer.

up to six weeks[29]. (M. Pfunder and B.A. Roy, unpublished), and many orchid species also have long-lived flowers[30,31].

The spatial distribution of species also influences competition for visitors and the likelihood of pollen transfer between them[14]. Plants that grow in intermingled clumps could attract more visitors as a result of their combined density, and the fact that each of the individual species grows in clusters might also increase the likelihood of proper pollen transfer[13,14]. However, it is also possible that clumped distributions might increase the ability of the pollinators to distinguish mimic from model and choose one over the other[32]. Experiments are needed to determine the relative importance of clustering in influencing pollinator behavior and to determine whether improper pollen transfer is reduced.

Competition favors character divergence and has thus sometimes been thought to be antithetical to the evolution of mimicry[2,8,32]. However, competition for visitors can lead directly to selection favoring similarity, if one species is favored over another, and if morphs that have greater similarity to the preferred species receive more visits and have higher pollination success and fitness (positive frequency-dependent selection, Fig. 1). Of course, selection can operate independently of phenotypic similarity for pollinator attraction and improper pollen transfer. For example, if visitation is density-dependent, and pollinators use visual cues for deciding where to forage, then selection will favor the convergence of flowers towards similar coloration, shape and size. Simultaneously, there might also be selection to decrease the degree of improper pollen transfer, and, thus, differences in the shape and length of anthers would be favored if they contacted pollinators' bodies in different places[33], or if pollen is scraped off on non-reproductive flower parts[34]. The outcome of simultaneous selection for visual similarity and differential pollen placement might yield something like the red, tubular-flowered guild that attract hummingbirds in the southwestern USA, in which the different species place the pollen on hummingbirds in different places. Indeed, this guild has been suggested to be a Müllerian mimicry ring[12], although not all of

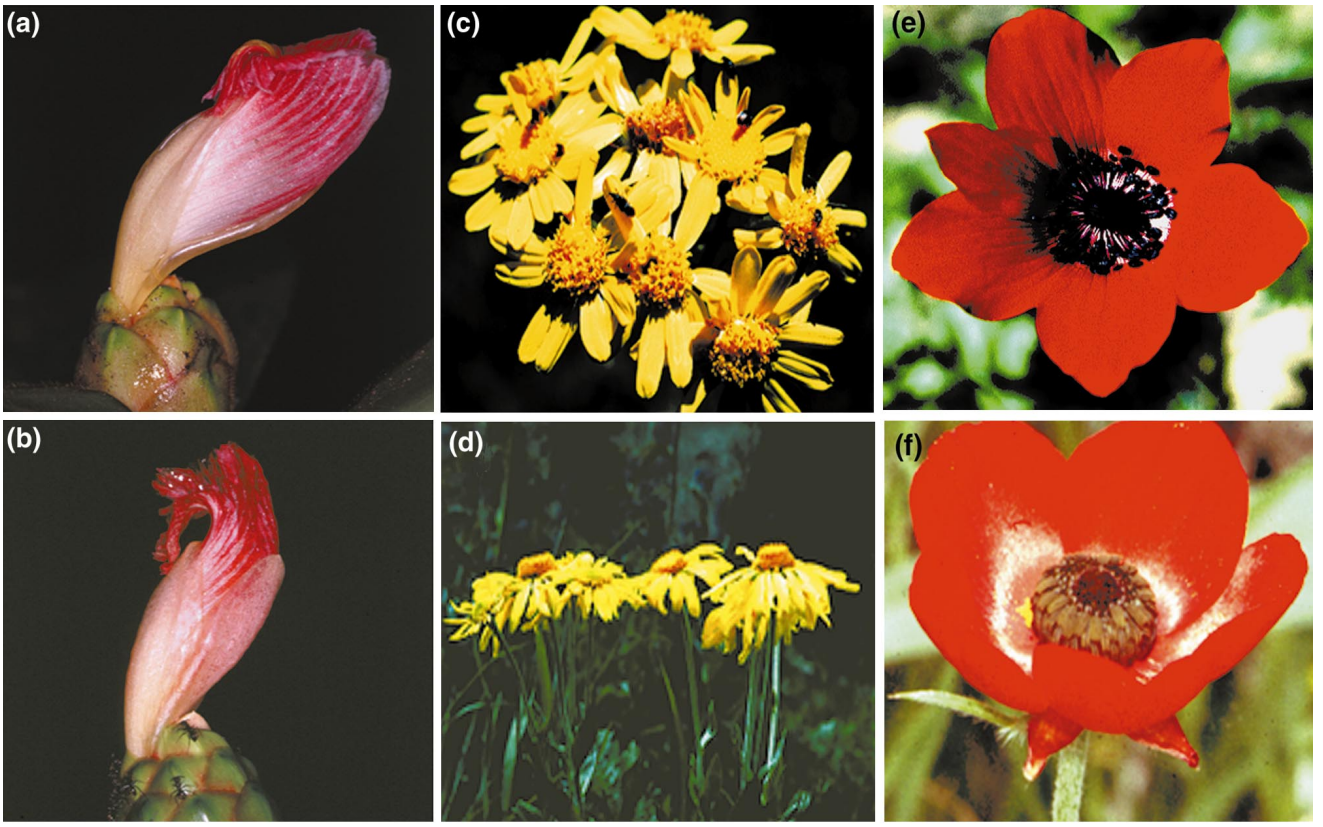

Fig. 3. Possible examples of Müllerian mimicry. (a) *Costus allenii* and (b) *Costus laevis* (Zingiberaceae) co-flower in central Panama. These species require visitation for full seed set, and share the same pollinator (*Euglossa imperialis*), which flies indiscriminately between flowers of the two species[7]. The flowers are nearly identical in color, morphology and nectar production, but they are nonetheless different species; the plants differ in vegetative characters and no hybrids form between them. Furthermore, recent molecular work has established that these are not closely related species, and thus their similarity is not the result of a close relationship (D. Schemske, pers. commun.). It is hypothesized that low flower density, which is compounded by high levels of flower predation, combined with a density-sensitive pollinator, selected for similarity between these species[7]. Unfortunately, seed set in single species versus mixed species plots has not been measured, so it is not known whether the similarity between these species facilitates reproduction. Equal visitation rates in mixtures might not always add up to the fitness achieved by individuals in pure patches because improper pollen transfer between species can reduce seed set[21–23,54]. Photographs (a) and (b) by Doug Schemske. (c) *Senecio integerrimus* and (d) *Helenium hoopesii* (Asteraceae) co-flower in the Rocky mountains along with numerous other yellow-flowered Asteraceae. The same insects visit all of these species and fly freely between them, visiting individuals in mixtures at the same rates as they visit individuals in monospecific patches[13]. A similar pattern has also been found for two species of *Potentilla* (*P. fruticosa* and *P. gracilis*)[13]. Unfortunately, seed set was not measured in single species and mixed species plots, so it is not known whether the similar visitation rates in mixtures led to a similar fitness as for the single species plots. Photographs (c) and (d) by Bitty A. Roy. (e) *Anemone coronaria* (Ranunculaceae) and (f) *Ranunculus asiaticus* (Ranunculaceae) co-flower in Israel along with red-flowered *Papaver rhoeas* (Papaveraceae) and *Tulipa agenensis* (Liliaceae). These species might form a Müllerian mimicry ring. These taxa are primarily pollinated by scarabaeid beetles[41]. All of the flowers in the red-flowered 'poppy guild' are bowl-shaped, have nearly identical color reflectance and a dark spot at the center. Interestingly, many of the species in this guild come from families and genera in which red is not a common color. Although there are differences in flowering phenology and in species of beetle visitors, there is considerable overlap of flowering time, and beetles do fly between plant species. It has not been experimentally determined whether the beetles forage in a density-dependent fashion, and no one has looked at seed set in mixed versus monospecific plots. Photographs (e) by Alex Widmer, (f) by Alexander Kocyan.

the critical tests of Müllerian mimicry suggested here have been performed. Another incompletely tested hypothesis suggests that flowers (and fungi) might sometimes attract visitors with visual cues, which allows the evolution of visual similarity, but simultaneously produce different fragrances that might influence insect constancy at close range[35,36]. This fragrance mechanism, if found to function, would be similar to mimetic butterfly species that converge on similar warning patterns, but which use species-specific pheromones for mate finding.

Competition is commonly cited for causing differences in flowering phenologies[21,37,38] (but see Ref. 39 for an alternative view). Although species that flower at different times will partition the available pollinators among them, species pairs that are similar to one another might gain an advantage early in their flowering seasons when they overlap because there will be no 'lag' period as pollinators adjust to a new morphology[40]. This mechanism might operate in the red-flowered, beetle-pollinated guild in Israel[41] and in co-occurring yellow-flowered composites[40] (Fig. 3).

Areas for future research

Although Müllerian mimicry has been suggested for several species of similar appearance that share pollinators, no one has performed the crucial tests to determine whether the similarity is adaptive (Fig. 3). If none of the putative cases of Müllerian mimicry is a true example, then this suggests that improper pollen transfer is a severe constraint on the evolution of floral mimicry. However, we suspect that experimental evidence will soon establish Müllerian mimicry as a fact in the plant world.

The two major kinds of mimicry are governed by different types of selection – negative or positive frequency-dependent selection (Fig. 1). These two kinds of selection allow us to make predictions about the degree of morphological variation that one should expect to see in different kinds of mimicry systems. In Batesian floral mimicry, where no reward is offered by the mimic, a large amount of phenotypic variation is to be expected because the more common a mimetic phenotype is, the more it will be actively avoided by pollinators[9,42]. For Müllerian floral mimicry, the opposite is true. Little variation in the mimetic phenotype is to be expected because the commoner the phenotype is, the higher its fitness is, and this kind of positive frequency-dependent selection ultimately leads to fixation of the most common phenotype (Fig. 1). Although these statements about polymorphism under the two kinds of selection are generally expected to be true, research on mimicry in butterflies has found the patterns of variation to be the opposite of expectations (reviewed in Ref. 19). We currently have too little information on the degree of phenotypic variation in flower mimics to make any general statements about patterns, although it appears that orchids might support the predicted pattern for Batesian mimics. Considerable morphological variation has been reported in orchid species that have been suggested to be Batesian mimics (reviewed in Ref. 9). Again, this variation is expected to be adaptive, because variation in the mimic will reduce the ability of pollinators to learn to avoid non-rewarding flowers[43,44].

Botanists lag far behind their zoological counterparts in terms of understanding the genetics of mimicry. We have no solid understanding of any traits involved in floral mimicry systems, and thus cannot test models suggesting that the evolution of mimicry is a two-step process[45]. Furthermore, there are no tests of basic phylogenetic hypotheses concerning mimicry. For example, does deceptive pollination always evolve from reward pollination systems[25,43]? Another obvious phylogenetic question, as yet untested in floral mimicry, is do species in Müllerian rings show evidence of co-evolution?

Finally, the importance of the 'signal perceiver' or 'operator' should not be forgotten in the evolution of mimicry because the habits and perceptual biases of the pollinators are crucial. It is intriguing to note that many of the pollinators implicated in potential Müllerian mimicry systems are trapline-pollinating euglossine bees or hummingbirds[7,12,46]. Trapliners (pollinators that visit the same plants daily) frequently include many species in a foraging bout[25], and are sensitive to floral density[7,46]. On the other hand, most flower visitors of deceptive Batesian mimics in the Mediterranean region are solitary bees[5,20,47,48].

The more we understand about what pollinators perceive, the better we will be able to identify the causes and consequences of mimicry. A critical issue is the degree of pattern recognition and resolution by pollinators. Flowers appear differently to insects than they do to us[49,50] and different pollinators might react to different stimuli[50,51]. We have concentrated on visual mimicry here, and because we are a visually oriented species we are more likely to observe visual mimicry. However, we should remain aware of the possibility for other characters, such as fragrances, to show mimicry.

Floral mimicry is a dynamic evolutionary process involving the perception of pollinators and the signaling capabilities of plants and some fungi. There is no reason to expect that selection for mimicry will be the same from one season to the next, or from one geographic area to another. Experimental approaches replicated throughout the flowering season and across geographic ranges are required to study floral mimicry, yet they have almost never been applied. Thus our understanding of floral mimicry remains in its infancy.

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technical focus

Caged peptides and proteins: new probes to study polypeptide function in complex biological systems

Now that the genomes of *Saccharomyces cerevisiae* and *Caenorhabditis elegans* have been sequenced, and the sequencing of the *Arabidopsis* genome is well under way, cell biologists are faced with the daunting challenge of establishing the function and mode of action of thousands of gene products. The ultimate goal of these studies will be to understand how the collective functions of these proteins and other biomolecules are responsible for life itself. Among the new tools that need to be developed to meet these challenges are optical probes and imaging technologies: these must be capable of identifying and mapping the interactions and activities of specific proteins and measuring their associated kinetic parameters with high spatial and temporal resolution. Light-directed activation of caged (inactive) compounds should be a valuable technique for such investigations because it can be used to manipulate the activity of specific biomolecules in cells (for a comprehensive review of the methodology involved see Ref. 1). This technique involves a biologically inert, caged compound that is specifically and rapidly (ns to ms) perturbed within a cell using a pulse of near ultraviolet light. The effects of a controlled and local (sub-micron) concentration or activity jump can then be recorded using time-resolved imaging of a probe of the perturbed reaction. Specific modulation of protein activity with light can initiate a specific cell process that might be used to elucidate the function of the protein, the kinetics of its activity and the mechanism of its action (for example, activation of myosin at the cell cortex and motility).

Over the past 20 years several caged ligands and substrates have been used to investigate the molecular basis of intracellular processes, including muscle contraction[2], synaptic transmission[3], intracellular signaling[4] and motility[5]. Unfortunately, small ligands often activate multiple cellular processes, or can be metabolized to other active species. Caged conjugates of peptides and proteins are often better suited for manipulating the activity of a specific protein within a cell[6,7]. The principle of light-directed activation of caged polypeptides, and the potential applications of this technique for studying the function of specific proteins in cellular processes are illustrated in Fig. 1.

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