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## Floral mimicry by a plant pathogen

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**INSECTS can effect sexual reproduction in some plant pathogens, such as the rust fungi, by carrying spermatia (gametes) between different mating types<sup>1–5</sup>. This function of insects is analogous to their role as pollinators of plants, and contrasts with their more widely known<sup>5–9</sup> role as vectors of plant pathogens' infectious spores. Here I report an extraordinary case of pathogen-mediated floral mimicry that contributes to fungal reproduction. The rust fungus *Puccinia monoica* inhibits flowering in its host plants (*Arabis* species) and radically transforms host morphology, creating elevated clusters of infected leaves that mimic true flowers of unrelated species in shape, size, colour and nectar production. These fungal pseudoflowers attract insects which fertilize the rust. Because the pseudoflowers are highly successful in attracting pollinating insects, they may also affect the reproductive success of nearby flowering plants.**

Species of *Arabis* (Brassicaceae), and a few other herbaceous mustards, become infected in late summer by wind-borne spores of the rust *Puccinia monoica* Arth.<sup>10,11</sup> Over the winter, rust mycelium invades the hosts' meristematic tissue, causing a systemic infection that affects all future growth. The rust fungus radically alters host morphology (Fig. 1). For example, infected plants of *A. holboellii* have twice as many leaves ( $41 \pm 2.8$  versus  $21 \pm 1.1$ ,  $Z = 7.72_{115,116}$ ,  $P < 0.0001$ ), twice as many leaf rosettes ( $3 \pm 0.4$  versus  $1.5 \pm 0.1$ ,  $Z = 5.41_{116,116}$ ,  $P < 0.0001$ ), and are twice as tall as non-flowering uninfected rosettes ( $97 \pm 5.9$  versus  $46 \pm 2.1$ ,  $Z = 5.37_{74,91}$ ,  $P < 0.0001$ ). Whereas uninfected plants form simple basal rosettes (Fig. 1a), infected plants form elongated stems crowned by dense, flower-like clusters of bright yellow infected leaves covered with a sticky, sweet-smelling exudate which is highly attractive to insects (Fig. 1c–e). Infected rosettes (pseudoflowers, Fig. 1c) bear little resemblance to uninfected rosettes (Fig. 1a), or to the flowers of host plants (Fig. 1b).

Pseudoflowers resemble unrelated flowers in form (Fig. 1e), colour (Figs 1e and 2), sweet-smelling odour, and sugar content (Table 2). Pseudoflowers often contain as much, or more, sugar than co-occurring flowers (Table 2) and other insect-pollinated flowers in similar habitats<sup>12,13</sup>. The bright yellow surface of the infected petal-like leaves (Fig. 1c–e) is largely composed of spermatogonia filled with spermatia, receptive hyphae, and sugary spermatial fluid. The colour of pseudoflowers is indistinguishable in both the ultraviolet and visible spectra from that of yellow flowers of some co-occurring angiosperms and contrasts sharply with green vegetation (Fig. 2). Yellow is a particularly common colour for flowers of plants that, like *Arabis*, frequent high elevations, high latitudes<sup>14</sup> and open habitats<sup>15</sup>.

Rust pseudoflowers attract a wide variety of flower-visiting insects including bees, butterflies, and flies (Fig. 1d, Table 2). Most rust fungi have outcrossing mating systems (are heterothallic) and require insect visitation for sexual reproduction<sup>1,2,5</sup>. I used an insect exclusion experiment to test whether *P. monoica*, like other rusts, requires insect visitation for sexual reproduction (Table 1). Sexual spores (aeciospores) only formed on infected plants that were 'open-pollinated', or in cages that included insects. Infected plants from which insects were excluded remained yellow and continued to produce nectar, whereas those that were visited made sexual spores, turned green, and stopped producing nectar. Similar colour changes are often observed after pollination in some true flowers<sup>16</sup>.

Flies, which accounted for most of the visits to rust pseudoflowers at field sites in Colorado (Table 2), have been shown to be effective carriers of rust spermatia<sup>1,2,17</sup> and are also important pollinators of montane and alpine flowers<sup>18,19</sup>. Crab spiders and other predators of pollinators were also observed waiting for prey inside pseudoflowers, just as they do in flowers. The floral mimicry fools humans as well as insects: botany students at the Rocky Mountain Biological Laboratory have frequently collected pseudoflowers thinking they were flowers and, at a distance, many professional botanists have mistaken them for true flowers.

Insect visitation to fungal pseudoflowers accounts for a substantial fraction of pollinator activity in natural communities (Table 2). Flies, the most common visitors to both pseudoflowers and *Pulsatilla*, stayed 5 times longer per visit on pseudoflowers, with an average visit length of 102 seconds ( $n = 46$ ) versus 21 seconds ( $n = 43$ ) on *Pulsatilla*. Insect visitors also spent 88% of their time on pseudoflowers in mixed plots including *Claytonia lanceolata*, *Ranunculus inamoenus*, and *Mertensia fusiformis* (Table 2). Pseudoflowers may detain pollinators for relatively long visits because spermatial sugar is spread over the whole surface of the pseudoflower, rather than being concentrated in a nectary. Rust pseudoflowers that detain insects for long periods may promote their own fertilization. Insects can bring opposite mating types together without moving between pseudoflowers if both fungal mating types are found on the same host plant, as commonly occurs in multiple-spore infections by wind-borne pathogens<sup>2,20</sup>.

By attracting pollinators, pseudoflowers may also affect the reproductive success of co-occurring flowering plants. Table 2 suggests that pseudoflowers could compete for pollinators in a way similar to that documented in some flower mixtures<sup>21–23</sup> because insects may spend most of their time visiting pseudoflowers rather than true flowers. Alternatively, rust pseudoflowers could facilitate pollination of co-occurring flowers by increasing the total 'floral' display or by providing an additional food source. For example, when rust pseudoflowers and the buttercup *Ranunculus inamoenus* occur together (Fig. 1e), visitation to both the buttercups and pseudoflowers is increased (B.A.R., manuscript in preparation). The influence of pseudoflowers on pollination of adjacent plants will depend on several factors, including the relative density of infected *Arabis*, the extent to which pollinators are shared between pseudoflowers and flowers, and the breeding systems of those co-occurring

TABLE 1 Effect of insect visitation on rust fungus (*Puccinia monoica*) reproduction

Treatment	Pseudoflowers producing aeciospores	Pseudoflowers without aeciospores	N	Likelihood ratio G*	P
Uncaged, 'open-pollinated'	20	0	20	53.59	<0.001
Caged, no pollinators	0	20	20	6.63	<0.01
Caged, 'dirty flies'	5	15	20	0.05	NS
Caged, 'clean flies'	3	17	20		
Overall test	28	52	80	63.74	<0.001

*Puccinia monoica* was unable to reproduce when insects were excluded. Not all infected plants that were visited by a single spore-covered fly produced spores, suggesting that: (1) the spermatia were incompatible, or (2) some caged flies did not visit the infected plant before they or the spermatia died. Some of the caged infected plants visited by 'clean' flies also produced spores suggesting that: (1) more than one mating type may be present on the same plant, or (2) the fungus is self-compatible but requires insect visitation to move spermatia between spermogonia, or (3) the putative 'clean' flies were also carrying spores. Infected plants in a meadow near the Rocky Mountain Biological Laboratory, Gunnison County, Colorado, USA, were tagged before the fungus became sexually receptive. Tagged plants were randomly assigned to four treatments: uncaged 'open-pollinated', caged (insects excluded), caged with 'dirty flies' (caged with one spermatia-covered fly captured feeding on an infected plant), or caged with 'clean flies' (caged with one fly captured on real flowers in an area where no rust infection was found within 1 km<sup>2</sup>, and therefore presumed to be spermatia-free). The flies were introduced when the rust was sexually receptive; all flies died within 4 days. Cages consisted of chicken wire cylinders surrounded by Kleen Test plant sleeves; they excluded insects while allowing light, water and wind to pass through. Uninfected plants caged in this way grew and flowered normally, indicating that the cages did not affect plant growth.

\* Multiple comparisons were corrected for experiment-wide error rate of 0.05, and the likelihood ratios were corrected with the Williams correction for small sample sizes<sup>28</sup>.

plants (obligate outcrossers are most vulnerable to changes in pollinator behaviour).

The floral mimicry reported here is fundamentally different from previously reported pathogen effects on plant hosts. For example, although spermatial stages of some rust fungi produce sweet exudates and attract insects<sup>1-5</sup>, the spermatia usually do not cover such large areas of the host, and the pathogen-mediated changes in host morphology do not resemble flowers.

Similarly, mummy-berry fungus has been reported to attract insects by secreting sugars and altering the ultraviolet reflectance of blueberry leaves<sup>7</sup>, but the insects disperse infectious spores rather than gametes, and infection does not radically alter host morphology. Finally, anther smuts invade host flowers, and disperse infectious spores by way of pollinators<sup>6,8,9</sup>. By contrast, *Puccinia monoica* does not exploit its hosts' flowers; instead, it causes its host to construct a completely counterfeit flower, one

FIG. 1 Morphological transformation of *Arabis* by *Puccinia monoica* rust infection. *a*, Rosette stage of uninfected *A. holboellii*. Uninfected plants remain in the rosette stage for 2-5 years. *b*, Flowering stage of uninfected *A. holboellii*. Infection of this host almost always prevents flowering and is usually lethal; in 116 pairs of infected and uninfected plants, 71% of infected plants died without flowering (versus only 12% of the uninfected plants), and none of the infected plants set seed. *c*, Bolting stage of *A. holboellii* infected by *P. monoica*. Infected plants act like they are bolting (elongation of the stem before flowering), but elongation stops long before normal flowering height is attained and true flowering almost never occurs. *d*, *Polygonia zephyrus* (Nymphalidae) feeding on the spermatial fluid of *P. monoica* on *A. holboellii*. *e*, *A. drummondii* infected by *P. monoica* (right) strongly resembles *Ranunculus inamoenus* (left), in shape, size and colour in the visible spectrum.

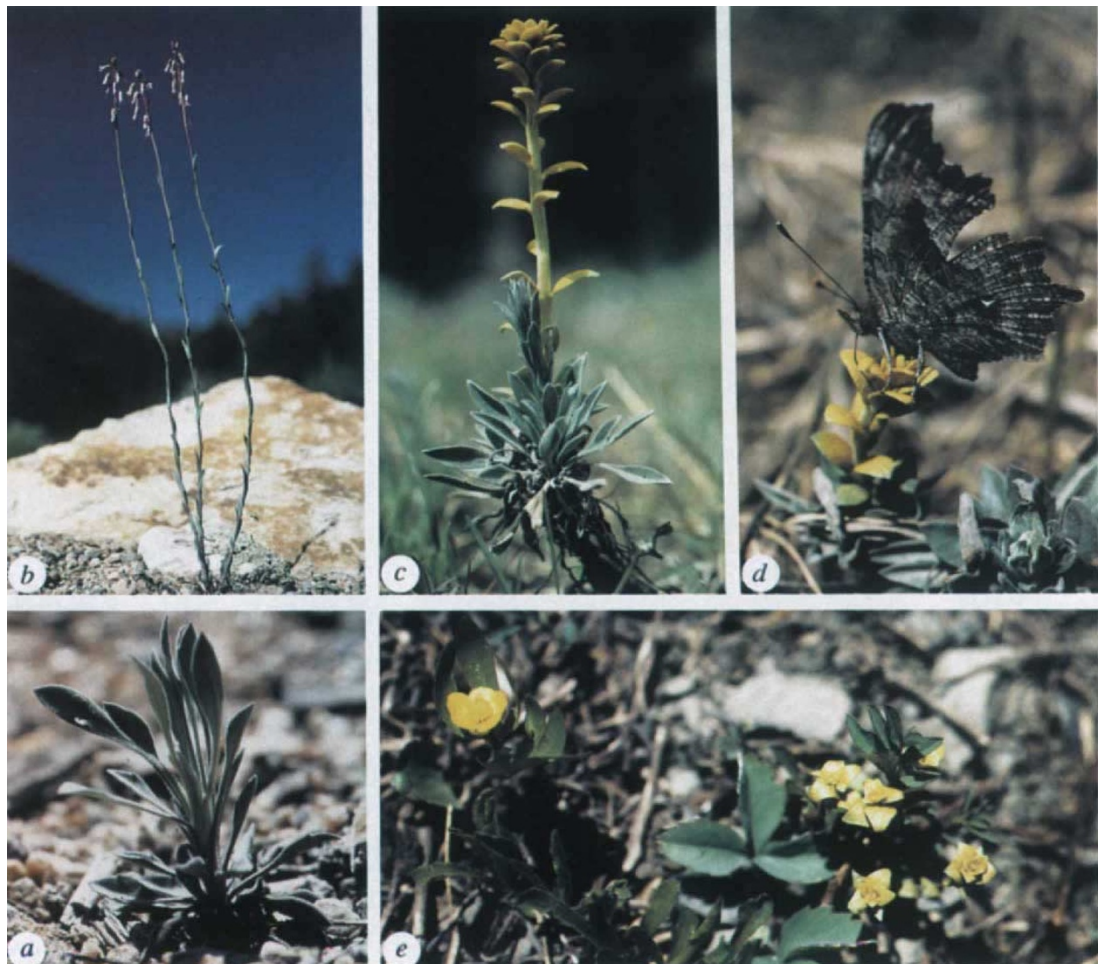


TABLE 2 Summary of flower sugar content and insect visitation to natural mixed plots of flowers including infected *Arabis* species

Plant species	Sugar per flower (mg)*†	Flowers in the plot (%)*	Visits to plots (%)	Time on plants (%)	Visits by flies (%)‡
Site one					
Rust pseudoflowers (on <i>A. holboellii</i> )	4.0 ± 0.70	52.0	45.1	72.2	100.0
<i>Pulsatilla patens</i>	0.12 ± 0.05	48.0	54.9	27.8	76.8
Site one total		n=98	n=102	108 min	n=89
Site two					
Rust pseudoflowers (on <i>A. drummondii</i> )	3.0 ± 0.32	34.2	37.0	87.9	90.0
<i>Claytonia lanceolata</i>	0.06 ± 0.01	2.3	0.9	0.03	33.3
<i>Mertensia fusiformis</i>	0.17 ± 0.02	47.1	9.9§	1.3	12.5
<i>Ranunculus inamoenus</i>	0.04 ± 0.01	16.3	52.2	10.7	14.8
Site two total		n=257	n=324	480 min	n=138

Insect observation took place between 11:00 a.m. and 3:00 p.m. during sunny, calm conditions. Site one consisted of 2.92 h of observation of three 1 m<sup>2</sup> plots near Almont in Gunnison County, Colorado (~2,700 m elevation). Site two consisted of 6.5 h of observation of three 1 by 2 m plots near Gothic, Gunnison County, Colorado (~2,957 m elevation). In all cases, insects were timed only when actually in the sexual parts of the flowers, or on the spermatial fluid of the rust fungus.

\* Individual pseudoflowers were considered to be flower analogues because they are similar in shape and size to many co-occurring flowers (Fig. 1e). For example, the diameters of pseudoflowers and the diameters of flowers of *Ranunculus inamoenus* and *Claytonia lanceolata* differ only slightly: 10.67 ± 0.39 mm, versus 11.47 ± 0.21 mm and 14.87 ± 0.29 mm, respectively, n=40. Sugar content data are typically reported on a per-flower basis whether the flowers occur individually or in inflorescences<sup>12,13</sup>. Here, single pseudoflowers are compared with single flowers. In the list above, only *Mertensia* has flowers in inflorescences.

† Sugar in spermatial fluid is fructose (identified chromatographically using Smith's solvent<sup>29</sup> of ethyl acetate, pyridine and water, and benzidine indicator reagent). Sugar content of 14 pseudoflowers per species was estimated by removing all infected leaves, soaking in distilled water for 1 h, and measuring sugar content of solution by refractometry. Sugar content of 20 flowers per species was estimated according to ref. 12; all sugar contents are reported as mean ± standard error, in sucrose equivalents.

‡ Fly visitors to infected *Arabis* include members of the Anthomyiidae, Bombyliidae, Calliphoridae, Muscidae, Stratiomyidae, Syrphidae and Tachinidae.

§ Actual insect visits to *Mertensia* could exceed observations; the inverted tubular flowers tended to hide visitors.

that mimics flowers of co-blooming species such as buttercups (Fig. 1e), rather than mimicking the host itself.

Rust fungi, including *Puccinia monoica*<sup>24</sup>, are well known for their ability to affect the evolution of their host populations through selection on resistance alleles<sup>25-27</sup>. My work indicates that some rust fungi, such as *P. monoica*, can also alter the growth and morphology of their hosts to a degree previously unknown. By redirecting host growth to produce strikingly flower-like forms, the fungus aids its own reproduction, alters the behaviour of insect pollinators, and possibly also affects the

reproductive success of co-occurring flowering plants. Thus this plant disease affects not only its hosts, but an entire natural community as well. □

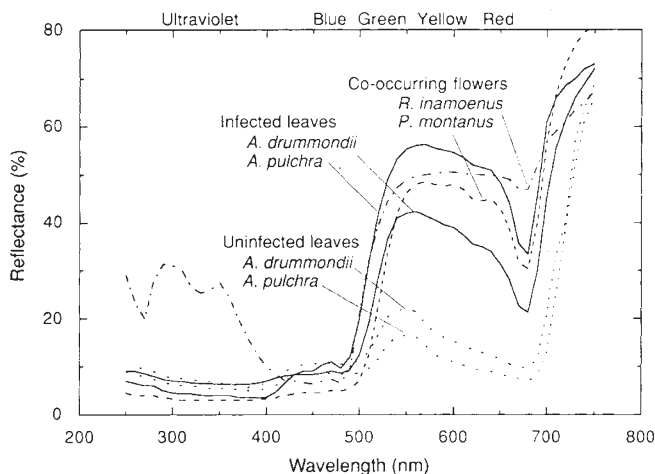


FIG. 2 Reflectance spectra of uninfected and infected *Arabis* leaves and petals of two co-occurring yellow flowers, *Ranunculus inamoenus* and *Pseudocymopterus montanus*. All spectra coincide in the visible spectrum; *Ranunculus inamoenus* also reflects in the near ultraviolet ('bee purple'). Leaves of *Arabis drummondii* were infected by *Puccinia monoica*, whereas leaves of *A. pulchra* were infected by *P. thlaspeos*. *P. thlaspeos* is very closely related to *P. monoica*<sup>30</sup> and also causes similar pseudoflowers to form on its hosts<sup>24</sup>.

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