the substrate or (ii) those tendons and apodemes where the maximum possible force on the tendon or apodeme is determined by maximum muscular contraction.

The use of safety factor and *TSF* described above can be generalized beyond load-bearing structures to make predictions about the relative performance of a much wider array of biological structures and systems by redefining safety factor as the ratio of average realized performance ($\bar{S}$) to average required performance ($\bar{L}_{mx}$) where, again, these averages are calculated for a population of individuals. For instance, one would predict that the average maximum hormonal output of a given type of endocrine gland would be greater, relative to the average threshold output required during stressful conditions, for populations whose maximum hormonal outputs or threshold requirements (or both) are more unpredictable. An analogous prediction would be that the average nectar output of a given flower type would be greater, relative to the zero variance output required to attract pollinators away from another population, for populations whose nectar outputs are more unpredictable. There is already empirical evidence that bumblebee foraging decisions could provide the selective pressures required for this prediction (*12*).

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7. A force (measured in newtons) was applied, with a Monsanto (type W) tensometer, in a dorsal direction perpendicular to the plane of the aperture by a steel hook of circular cross section (1.16 mm in diameter) inserted under the edge of the shell to mimic the prying action of a rounded crab appendage; the breaking force did not differ significantly in pairwise comparisons among hooks ranging in diameter from 0.67 to 2.64 mm (smallest $P > 0.15$, $n = 7 - 20$; analysis of covariance).
8. Spring scales were used to load (force measured in newtons) the edge of the shell in a manner identical to and on the same sides as the breaking force measurements. Limpets from Lime Kiln Lighthouse (all except *N. persona*) and False Bay (*N. persona*) on San Juan Island, Washington, were used both for strength and tenacity measurements.
9. Model 1 analysis of covariance was used to calculate *TSF* and shell strength variance (data were transformed to natural logarithms). For each species and side of the shell, I calculated regression equations for breaking force as a function of foot area ($n = 7 - 20$) and maximum tenacity as a function of foot area ($n = 8 - 23$). All breaking force–maximum tenacity regression pairs were parallel (no statistically significant differences between the regression coefficients of each pair; smallest $P > 0.05$). Hence, the *TSF*'s were essentially constant over the size ranges tested. Therefore, $TSF = \exp(\ln \bar{S} - \ln L'_{mx})$ where $\ln \bar{S}$ was the adjusted mean of the breaking force regression and $\ln L'_{mx}$ was the adjusted mean of the maximum tenacity regression for each species and side of the shell. Shell strength variance equaled the residual variance (*MSe*) of the breaking force versus foot area regressions. The *MSe*'s are reported as coefficients of variation (*CV*) where $CV = [\exp(MSe) - 1]^{0.5}$ [P. N. Chalmer, *J. Zool. (London)* **191**, 241 (1980); R. C. Lewontin, *Syst. Zool.* **15**, 141 (1966); S. Wright, *Evolution and the Genetics of Populations* (Univ. of Chicago Press, Chicago, 1968), vol. 1].
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14. For each point in Fig. 3, ln *TSF* ± 1 standard error, and *CV* ± 1 standard error: *C. digitalis* A, 0.67 ± 0.147, 0.45 ± 0.108; *C. digitalis* P, 0.30 ± 0.161, 0.36 ± 0.100; *C. pelta* A, 0.46 ± 0.125, 0.46 ± 0.117; *C. pelta* R, 0.20 ± 0.128, 0.20 ± 0.051; *C. pelta* P, −0.15 ± 0.093, 0.22 ± 0.062; *N. scutum* A, 0.23 ± 0.086, 0.31 ± 0.054; *N. scutum* R, 0.08 ± 0.137, 0.27 ± 0.072; *N. scutum* P, −0.17 ± 0.102, 0.19 ± 0.050; *A. mitra* A, 0.66 ± 0.121, 0.33 ± 0.077; *N. persona* A, 0.21 ± 0.128, 0.31 ± 0.086; *D. aspera* A, 0.22 ± 0.134, 0.18 ± 0.047; A, R, P as for Fig. 3.
15. I thank R. McN. Alexander, A. A. Biewener, G. M. Branch, J. D. Currey, D. H. Kelker, M. LaBarbera, A. R. Palmer, C. Strobeck for their suggestions and the use of equipment, and two anonymous reviewers for comments. I also thank the staff and biomechanics group at the University of Washington's Friday Harbor Laboratories for providing research space and facilities. Funding was provided by a Killam predoctoral fellowship to R.B.L., NSERC operating grant A7245 to A. R. Palmer, and the Department of Zoology, University of Alberta.

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Floral Mimicry Induced by Mummy-Berry Fungus Exploits Host's Pollinators as Vectors

Abstract. *Leaves and shoots of blueberries (*Vaccinium *spp.) and huckleberries (*Gaylussacia *sp.) when infected by ascospores of* Monilinia *spp. become ultraviolet-reflective and fragrant and secrete sugars at their lesions. Insects that normally pollinate these hosts are attracted to the discolored leaves, ingest the sugars, and transmit conidia to their flowers, resulting in sclerotia (mummy-berry) formation.*

Monilinia vaccinii-corymbosi (Sclerotiniaceae), an economically important discomycete fungus, blights leaves and vegetative and floral shoots and mummifies fruit of wild and cultivated blueberries (*Vaccinium* spp.; Ericaceae); crop yield losses may reach 85 percent in individual fields (*1*). This polytrophic, dimorphic fungus overwinters on moist soil as sclerotia in mummified berries (or mummy berries). Unfolding young leaves of the host are infected by windborne, sexual ascospores released from apothecia, which arise from the sclerotia in early spring; these leaves (blight or wilt stage) produce asexual conidia that are transferred by pollinating insects to the host's flowers where the ovaries become infected, producing seedless, inedible mummy berries (*1*).

Various polyphagous insects that casually feed on exudates or spores of fungal plant pathogens are well known to disperse spores randomly (*2*). Azalea flower spot, caused by *Ovulina azaleae* (Sclerotiniaceae), is transmitted by pollinators that accidentally contact spores (*3*). We describe the behavior and role of insect pollinators of blueberries and huckleberries (*Gaylussacia* sp.; Ericaceae) in transmitting mummy-berry diseases. The exploitative modification of pollinator behavior through induction of floral mimicry in infected leaves by a vector-dependent, host-specific plant pathogen appears to be unique.

We investigated the interrelationships among 22 species of pollinating insects (*4*), *Monilinia vaccinii-corymbosi*, an unnamed *Monilinia* sp. (*1*), and their respective hosts, *Vaccinium corymbosum*, *V. vacillans*, and *Gaylussacia baccata*, between 1976 and 1984 in Greenbelt, Maryland (*1*). These sympatric wild hosts grow as understory shrubs in moist soil in a mixed oak-pine forest; most of their flowering is completed within 10 days, before the tree canopy fully leafs out. They have pendant flowers with poricidal anthers that require insect pollination (Fig. 1A) for fertilization (*5*). The earliest symptom of infection by *Monilinia* is wilting of young leaves and shoots, followed within 24 hours by browning of the upper side of the drooping shoots, midribs, and lateral veins of leaves (Fig. 1, B and C). Discoloration, which may spread to engulf the entire leaf, ranges (in daylight) from grayish brown to deep brown and dark brown, often noticeably to strongly tinged with moderate violet (*6*); in *Gaylussacia*, the discoloration is dark to moderate olive with a slight violet sheen (*6*). A grayish mantle of conidia, conidiophores, and occasional hyphae appears on the surface of infected shoots, peduncles, petioles, and at the base

of leaves along their midribs (Fig. 1D). An odor resembling fermented tea is produced by infected leaves. Insect species that characteristically pollinate the host plants are attracted to these wilted leaves (*4*), where they lick the mantle of conidia and become contaminated with spores (Fig. 1E). When these insects subsequently visit host flowers, the conidia are deposited on the stigmas, resulting in infected ovaries (mummy berries), and on other floral parts, to be further transmitted by additional pollinators. These short-lived (*1*) conidia require insects as vectors. When we bagged 400 hand-pollinated flowers of a blueberry plant on branches also bearing infected leaves, with fiberglass screening to exclude most insects but not wind, 10 percent mummified fruit was produced; whereas similarly infected, unbagged, open-pollinated branches of the same plant, at the same height, bore 63 percent mummified fruit.

The ratio of infected, wilted leaves to blossoms varies seasonally, according to rainfall, and is highest on low branches overhanging moist ground where apothecia are numerous. During their search for blossoms, insects are attracted to infected leaves in sun or shade at rates of 3 to 24 ($\bar{x}$ = 9) visits per hour (*4*). Their approach to wilted leaves, alighting, and search patterns (76 observations) resemble tactics used when encountering host flowers—for example, they zigzag, usually making upwind search flights, and alight atop objects (petioles of wilted leaves or calyces of flowers); they walk in rotation while seeking food, bringing the insect beneath the flower or in contact with conidia on the leaf; and they lick, ingesting floral nectar or conidia. We did not see bees make pollen-collecting motions when encountering conidia. They and the other vectors were never seen licking healthy leaves, petioles, and midribs. Although some varieties of *Vaccinium* have extrafloral nectaries on leaf margins, visits to such nectaries were not seen at the study site. Analysis by thin-layer chromatography of the conidial mantle, where most licking occurs, revealed that sucrose, glucose, and fructose are present. Nectaries of *Vaccinium* contain about 20 percent sucrose and 80 percent glucose and fructose (*7*).

In daylight, the wilted leaves do not visually resemble the host's white, yellow, and pink flowers; however, the pollinators behave as if they are visiting nectar-yielding flowers. Investigation (*8*) of discolored areas of infected leaves that appear brown to violet in daylight showed that these areas reflect ultraviolet light at 300 to 400 nm (Fig. 1F). Blueberry floral calyces are also ultraviolet-reflective, and they and infected leaves visually contrast with healthy, ultraviolet-absorbent (*9*) surrounding leafy vegetation. Thus, the *Monilinia*-infected, discolored leaves evidently mimic the flowers of their host in yielding sugary rewards to the pollinator-vectors and, to some extent, in providing them with ultraviolet-reflective patterns analogous to nectar guides.

Flowers of certain orchids and other vascular plants may falsely attract pollinators (*10*). The *Vaccinium* (or *Gaylussacia*)-pollinator-*Monilinia* complex is unusual because host-pollinator mutualism is exploited by host-specific and vector-dependent pathogens, which achieve their necessary dissemination and survival by causing "deceitful" floral mimicry (*11*).

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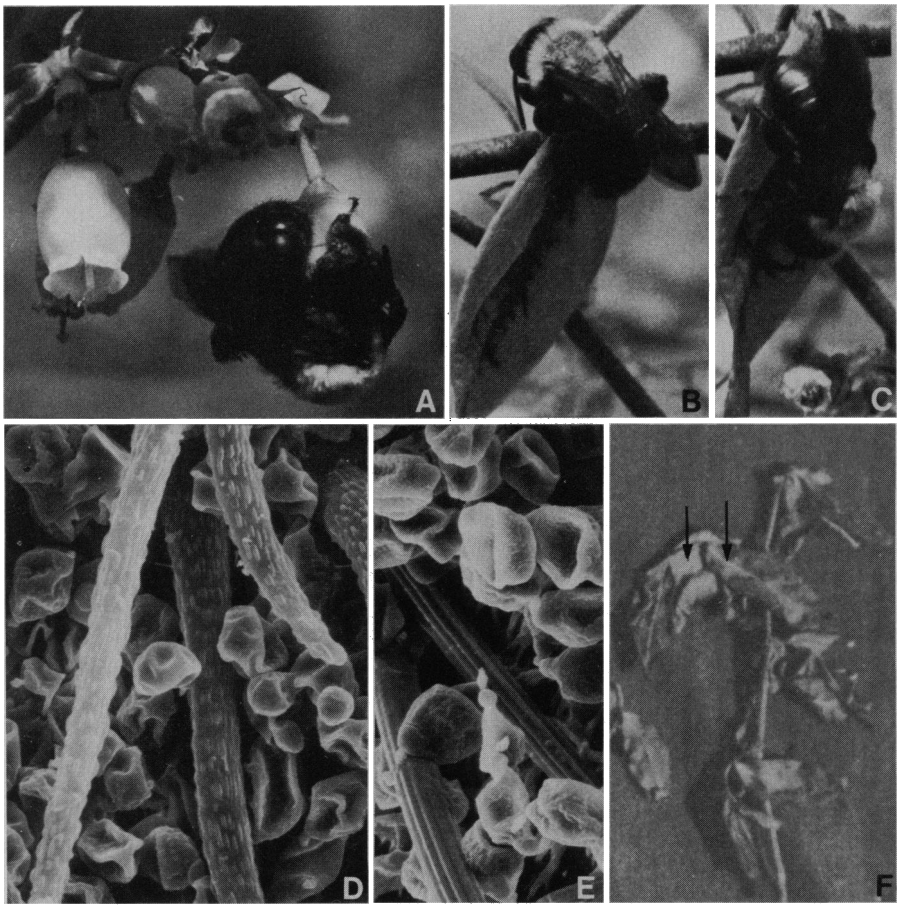

Fig. 1. Pollinators, the pathogen, and the host, *Vaccinium corymbosum*. (A) Female *Andrena carlini* pollinating a host flower. (B and C) Female *A. carlini* licking petiole and midrib of infected leaf (daylight illumination). (D) Conidia of *Monilinia vaccinii-corymbosi* on the midrib of an infected leaf amid plant hairs (×780). (E) Conidia among facial hairs of a pollinator, *Epalpus signifer* (×900). (F) Infected leaves as seen in ultraviolet light; discolored areas (arrows) that seem dark (absorbent) in daylight are light (reflective) under ultraviolet. (Photographed with a Wratten 18A filter, Nikon 55-mm *f*/3.5 glass lens and Tri-X film.)

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11. We also casually observed pollinators visiting wilted leaves of the following species infected by the respective fungi: *Monilinia amelanchieris* on *Amelanchier canadensis*, *M. johnsonii* on *Craetegus viridis*, *M. megalospora* on *Vaccinium uliginosum*, *M. oxycocci* on *V. macrocarpon*, and *M. polycodi* on *V. stamineum*.
12. We thank H. M. Fales for assistance with sugar analysis and N. Chaney for help with scanning-electron microscopy. Diptera and Lepidoptera were identified by R. J. Gagné, D. Ferguson, and F. C. Thompson. The cooperation of M. Hunt during several years is appreciated. E. M. Barrows, P. O. Batra, and R. W. Lichtwardt read the manuscript.

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Intracellular Stimulation of an Identified Neuron Evokes Cardioacceleratory Peptide Release

Abstract. *The central nervous system of the tobacco hawkmoth,* Manduca sexta, *is known to contain two cardioacceleratory peptides (CAP's), both of which function in vivo as cardioregulatory neurohormones. Intracellular electrical stimulation of a single abdominal ganglion neuron evokes the release of CAP-like bioactivity. This stimulation-evoked bioactivity is destroyed by prior treatment with protease. The possibility that intracellular stimulation of a CAP-containing neuron synaptically activated additional spiking neurons is eliminated.*

Neuropeptides in the central nervous system (CNS) are capable of acting as neurotransmitters (*1–3*) and as neurohormones (*4–6*). It is often easier to define a role for a neuropeptide if that neuropeptide can be unequivocally associated with an identifiable neuron or neurons. There are several physiological, anatomical, and pharmacological criteria that must be met before a neuropeptide can be established as a neurochemical mediator at the cellular level (*7*). Most of these criteria are similar to those for the rigorous identification of conventional neurotransmitters (*8*). One crucial criterion frequently overlooked is the demonstration that the neuropeptide is released when the putative peptidergic neuron is individually depolarized above threshold. Although peptide release from the CNS has been shown in several preparations by treatment with K^+-rich saline (*9–11*) or by electrical stimulation of peripheral nerve roots (*12–14*), it has been difficult to demonstrate peptide release resulting from the activity of single cells regardless of whether the neuropeptide is acting as a neurotransmitter or as a neurohormone. We show here that intracellular electrical stimulation of a single, identified neuron is sufficient to elicit the release of neuropeptide activity from its terminal endings.

We have studied the cardioacceleratory peptide (CAP) system in the tobacco hawkmoth, *Manduca sexta*. Earlier investigations (*15–17*) have shown that two cardioactive neuropeptides, known as cardioacceleratory peptide 1 (CAP_1) and cardioacceleratory peptide 2 (CAP_2), are present in the pharate adult ventral nerve cord (VNC). The two CAP's are coreleased into the hemolymph from the segmentally repeated transverse nerves (Fig. 1B) immediately after adult emergence, and they act to increase heart rate significantly and to facilitate inflation of

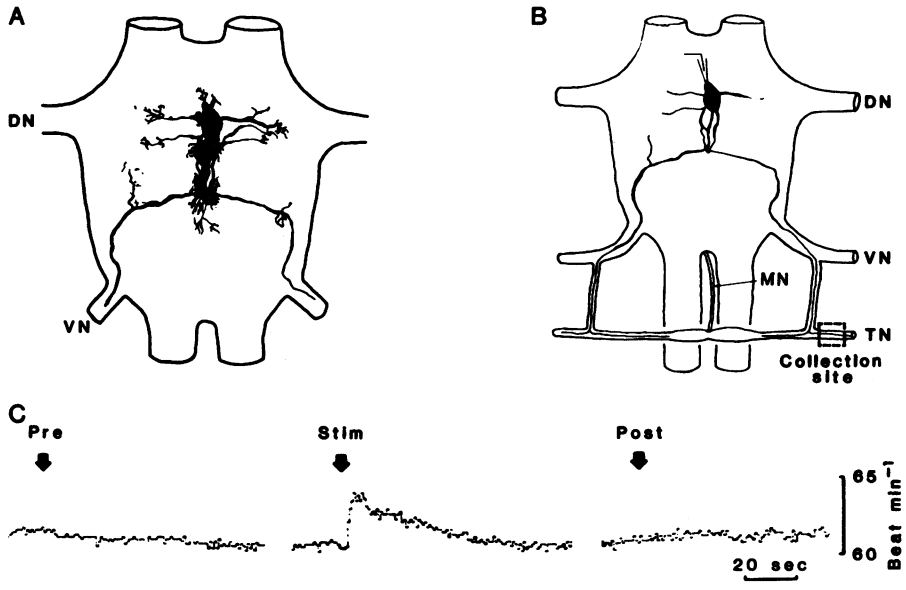

Fig. 1. Stimulation of a new MB neuron causes the release of CAP-like bioactivity. (A) Camera lucida drawing of a new MB neuron in a pharate adult abdominal ganglion. We stained the cell by passing positive current through an intracellular micropipette filled with 48 percent hexamine cobaltous chloride. In the preparation a modification of Timm's silver intensification procedure was used (*23*). The bifurcating axon exits the ganglion via both ventral nerves. The cell terminates bilaterally in neurosecretory endings along the length of the transverse nerve (*24*). (B) Diagrammatic representation of the experimental protocol. We impaled a soma of a new medial bilateral (MB) cell, using standard glass microelectrodes, and depolarized it by passing d-c current pulses for up to 15 minutes at a frequency of not greater than 0.5 Hz. Although not visible in situ, each new MB cell was identified unequivocally on the basis of its cell body position in the ganglion, the trajectory of its axons, and the characteristic electrical properties of its soma. As is typical of insect neurosecretory cells (*24, 25*), the somata of the new MB neurons were electrically excitable, capable of supporting overshooting action potentials with durations of approximately 50 msec. Thus, as a group these neurons were uniquely recognizable during recording sessions. As it proved impossible to maintain somatic activity with dye-filled microelectrodes, we were unable to distinguish between the two anteriormost pairs of new MB cells. We collected CAP activity by erecting a Vaseline well (volume ~0.1 ml) around the transverse nerve at a point distal to the transverse nerve–ventral nerve anastomosis. The contents of the well were collected at various times, frozen on Dry Ice, and stored at −20°C, usually for less than 24 hours, until bioassayed for CAP activity. Abbreviations: TN, transverse nerve; DN, dorsal nerve; VN, ventral nerve; MN, median nerve. (C) Cardioacceleratory activity of samples collected during intracellular stimulation of a new MB cell. Each sample was sequentially bioassayed on the same in vitro *Manduca* heart as described (*16, 17, 21*). For these experiments, the variability in the basal heart rate was ≤1 percent. Arrows denote application of samples. The heart rate increased after application of the Stim sample. A standard lepidopteran saline (*16*) was used in all experiments.