REVIEW

Dark Matters: Challenges of Nocturnal Communication Between Plants and Animals in Delivery of Pollination Services

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The night is a special niche characterized by dim light, lower temperatures, and higher humidity compared to the day. Several animals have made the transition from the day into the night and have acquired unique adaptations to cope with the challenges of performing nocturnal activities. Several plant species have opted to bloom at night, possibly as a response to aridity to prevent excessive water loss through evapotranspiration since flowering is often a water-demanding process, or to protect pollen from heat stress. Nocturnal pollinators have visual adaptations to function under dim light conditions but may also trade off vision against olfaction when they are dependent on nectar-rewarding and scented flowers. Nocturnal pollinators may use CO_2 and humidity cues emanating from freshly-opened flowers as indicators of nectar-rich resources. Some endothermic nocturnal insect pollinators are attracted to thermogenic flowers within which they remain to obtain heat as a reward to increase their energy budget. This review focuses on mechanisms that pollinators use to find flowers at night, and the signals that nocturnally blooming flowers may employ to attract pollinators under dim light conditions. It also indicates gaps in our knowledge. While millions of years of evolutionary time have given pollinators and plants solutions to the delivery of pollination services and to the offering of appropriate rewards, this history of successful evolution is being threatened by artificial light at night. Excessive and inappropriate illumination associated with anthropogenic activities has resulted in significant light pollution which serves to undermine life processes governed by dim light.

INTRODUCTION

The night is a special niche that is characterized by the absence of the sun as a source of energy and light but the presence of other entities such as the moon and the stars that provide light and environmental cues. Nocturnal organisms have to cope with the sun's absence and evolve ways of using alternative celestial bodies. Therefore, the night is occupied by organisms with special functional traits. Since the sun and its photons have played a fun-

damentally important role in influencing life on Earth, organisms that have opted to function in the absence of the sun still carry the signatures of evolution under solar influence [1], as well as some unique adaptations to life with only the moon and stars for guidance [2]. The initial use of light energy to drive proton pumps across membranes, coupled with energy capture and transfer, evolved early on in microbes with the evolution of transmembrane rhodopsins with retinal as the light-accepting chromophore; light was then co-opted as a signal to per-

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ceive surroundings, to navigate, and to move towards [3,4]. The fundamental role and evolutionary history of ubiquitous chromophores such as retinal cannot be underestimated in this harnessing of energy quanta for various purposes [4]; these chromophores were later co-opted for vision [3]. Light is now an important and reliable environmental cue that governs a host of physiological activities in plants and animals that range in scale from days through months and years. Feeding, light harvesting for autotrophy, mating, development, short- and long-range movements, and myriad other activities are facilitated by light [5,6].

This review will address the principal constraints and new adaptations that characterize the interactions between nocturnally blooming plants and their nocturnal pollinators. It will attempt to provide an evolutionary perspective on the shift to night activity for both partners in such mutualistic interactions, and will also briefly examine the consequences of the presence of artificial light at night (ALAN) for the future of such interactions.

FEATURES OF THE NIGHT

The night has very special features that result in it being characterized as a unique niche for pollination [7]. On a moonless night, light levels are 100 million times lower than those of a cloudless, sunny day [8], while on a full moon night, light levels are approximately 1 million times dimmer than full daylight. Light provided by starlight on a moonless night is about 100 times dimmer than that of a full moon night [9], while a quarter moon provides light about 10 times less, and the light of a crescent moon is not much more than that provided by the stars [10]. Clouds in the sky or light under a forest canopy at night can reduce light levels by another 100 times [11]. While the color of light that filters through a forest during the day has been investigated [12], that of a forest in the night has scarcely been examined. However, Madagascan forests and woodlands have a yellow–green-dominant nocturnal light environment [13]. Furthermore, while moonlight has a color spectrum similar to that of sunlight, starlight is red-shifted towards longer wavelengths [9]. These reductions in light levels and shifts in color spectrum indicate that using vision at night is a challenging task. Furthermore, wind speeds and temperatures tend to be lower in the night compared to the day while humidity levels are often higher [7]. This means that nocturnal pollinators have to find floral resources under low-light conditions, may have to perform this function at low temperatures, and may not be able to use wind-assisted flight to move between flower patches.

POLLINATORS IN THE NIGHT: OVERCOMING THE CHALLENGES OF THE NIGHT

Taxa of Nocturnal Pollinators

Despite the diversity of organisms that are active in the night, only few groups perform pollination services. Of these, the insects are the most important, but even within the Insecta, only some groups such as moths, beetles, bees, and dipterans such as fungus gnats and gall midges are important [7]. Among vertebrates, bats are undoubtedly the most prominent, while rodents, primates, and some smaller carnivores such as civets are also important as pollinators [7]. While there are several nocturnal birds, none are known to be pollinators. Among reptiles only geckos are nocturnal pollinators, and that too mostly on islands where other nocturnal taxa are missing from the biota [7]. Why only some pollinator taxa function in this nocturnal niche is a likely consequence of evolutionary history as well as of constraints on adaptations to a nocturnal lifestyle.

Vision in the Night

In terms of vision, the best studied representatives of nocturnal pollinators are invertebrates such as bees, hawkmoths, and a few vertebrates such as geckos [14,15]. The principal problem that all these eyes have to solve is to increase photon capture and processing because the greater the number of photons captured, the greater the potential light signal that can guide a behavioral output. The solution can be achieved in different ways and at different levels, and involves compromises and trade-offs. The problem can be solved at the peripheral light gathering or photoreceptor level stage, and at the central processing stage within the nervous system.

Peripheral stage: A) Eye design: Among nocturnal insects, such as moths, compound faceted eyes have a superposition design in which light entering through multiple facets can converge onto the retina resulting in signal amplification. Diurnal insects, on the other hand, need to protect the retina from too much light; hence diurnal apposition eyes have pigment shields between facets that guide light only through a single facet onto the retina; consequently, there is no signal amplification at this stage [15]. Nocturnality also selects for a larger number of photoreceptors that can only be accommodated in larger eyes. Nocturnal carpenter bees, for example, which have inherited apposition eyes from diurnal ancestors, have larger eyes with larger facets than diurnal ones [7,16]. These large facets also often have shorter focal lengths to ensure as little light loss as possible from the moment of light entry into the eye until signal generation. Such adaptations ensure that, for example, the apposition

eyes of the crepuscular owl butterfly are four times more sensitive than the apposition eyes of the diurnal *Morpho* butterfly [17]. While much is known about electrophysiology and the limits of phototransduction in apposition eyes such as those of flies and bees, electrophysiology in superposition eyes such as in moths has proved to be notoriously difficult and, therefore, such eyes have largely been investigated at the level of neuroanatomy [18]. Consequently, most knowledge about vision in dim light comes from apposition eyes. However, optics do matter in the functionality of eyes; this is why a nocturnal butterfly with an ancestral apposition eye design has evolved superposition optics analogous to those of nocturnal moths [19].

B) Photoreceptor properties: To use the energy of light as a visual, navigational, or phototactic guide, photoreceptors have to deal with three types of noise: photon shot noise, physiological noise or transducer noise, and dark noise [15]. Photon shot noise is the random variability, best described as a Poisson process, in the arrival of photons for capture by photoreceptors; obviously the lower the light levels, the greater the variability in photon arrival and in the signal to noise ratio. A larger number of photoreceptors would result in a less variable signal, which would therefore select for many more photoreceptors in nocturnal eyes compared to diurnal ones. Despite this photon shot noise, all photoreceptors are able to respond to single photons of light, each of which can induce what are referred to as quantum bumps, *i.e.,* the depolarizing or hyperpolarizing current induced by light quanta. Physiological noise or transducer noise is the variability in the current that is generated because of variation in the exact response of the receptor transduction machinery to each photon. Despite transducer noise, the quantum bumps in the photoreceptors of nocturnal sweat bees and spiders are much larger (5 to 20 times) with longer durations and integration times (therefore slower responses) compared to diurnal sweat bees or flies [15,20]; this indicates that electrical amplification is much greater in nocturnal photoreceptors than diurnal ones for the same stimulus. Many other nocturnal pollinating insects such as cockroaches and crane flies also demonstrate large quantum bumps in their photosensing machinery [18]. Dark noise is the result of the spontaneous thermal activation of opsins; this can mimic the presence of a light signal when none exists, and is also the reason why infra-red light, which has more thermal energy that other regions of the visual spectrum, is not suitable for vision [21]. In vertebrates, dark noise fixes the threshold for vision under dim light conditions; however, dark noise is apparently not so important in insect photoreceptors [11].

Photoreceptors throughout the animal kingdom are either of the microvillar or ciliary type [22]. Microvillar photoreceptors are dominant in most invertebrates pos-

sibly because they are more sensitive when compared to ciliary photoreceptors and can respond to single photons [22]. Vertebrate eyes have duplex retinae comprised of ciliary rods and cones that respond to achromatic and chromatic (color) signals respectively; rods are slow in their response but have high sensitivity while cones are fast responders with low sensitivity [22]. Rods are efficient in scotopic or dim light vision for a variety of reasons. First, the packing density of opsins in a rod is 10 times higher than that in microvillar insect receptors [22]. Second, rods are more energy efficient than microvillar receptors over a range of light conditions. Cation channels in rods are kept open in darkness by the binding of cGMP [23]. Light stimulates the hydrolysis of cGMP resulting in channel closure, and the light-induced current causes hyperpolarization. Since in most vertebrates, including mammalian pollinators, bright light closes rod channels, energy consumption in the light is decreased by > 75 percent [24]; in diurnal insects such as *Drosophila* with depolarizing microvillar photoreceptors, light opens cation channels with a five-fold increase in energy consumption over dark conditions [24]. Therefore, the evolution of rods has conferred an energetically efficient advantage of considerable magnitude under dim light conditions; this has perhaps been achieved at the cost of color vision which has been transferred to cone receptors in the duplex retinae of vertebrates.

Central or Higher-order Processing Stage: Spatial and Temporal Light Signal Summation

Spatial summation is analogous to printing an image with a dot matrix printer at a low dpi (dots per inch) value; *e.g.,* 300 dpi provides a fuzzier image with coarser resolution compared to an image printed at 600 dpi. Temporal summation is analogous to taking a photograph with the aperture of the camera lens kept open for a long time (the time over which the light/images are superimposed); this will result in visual information being made available at slower time frames. The advantage of spatial and temporal summation in visual systems is that the behavior of the organisms is now governed by centrally processed input that has acquired signal gain since the input exceeds the information provided by individual photoreceptors [25]. The trade-off resulting from summation is that nocturnal eyes may see blurry images that are resolved at slower speeds; this could also affect the speed of locomotion and the tracking of moving resources [26]. Signal summation in some form is a general response to low light; even diurnal birds show behavioral evidence of spatial summation under dim light conditions [27].

Evidence for neural spatial summation in nocturnal eyes has been gathered in a comparison of diurnal and nocturnal hawkmoths [28]. In this study, while the lateral dendrites of type 2 and type 4 neurons in the lamina (the

region of the insect brain just after the retina) of nocturnal hawkmoths were found to have the lateral arborization and anatomical capacity to connect with neighboring neurons, and thus to effect spatial summation, only type 2 neurons actually have this capability. The eyes of diurnal hawkmoths, on the other hand, had low summation capacity. While hawkmoth eyes do not have the duplex retinae of vertebrates, the eyes of day-active species have faster temporal tuning (analogous to rods) while those of nocturnal species have slower temporal tuning (analogous to cones) [29]. Neural summation in hawkmoths can allow motion detection at illumination levels 100 times dimmer than that achievable only by adaptations in the optical features of their eyes [30].

Since spatial resolution is lower in the eyes of nocturnal and crepuscular hawkmoths [28], how nocturnal species track swaying flowers so that they do not overshoot or undershoot them, while hovering to extend their proboscis for feeding, is an interesting problem. Using robotic flowers, the visual system of the nocturnal hawkmoth *Manduca sexta* was found to slow down in dim light; moths performed worse in tracking flower movement in dim moonlight compared to dusk light levels [26]. In an analysis of diurnal, crepuscular and nocturnal hawkmoths, all species demonstrated illumination-dependent delays in tracking the movement of artificial flowers [31]. Could these constraints have imposed selection on the biomechanical properties of flowers to prevent them from swaying or swinging too much in wind, especially when they bloom at night? This is an interesting question [32] that remains to be answered.

Nocturnal *M. sexta* also has an innate preference for blue flowers which can be modified by foraging experience and foraging conditions such as variation in ambient illumination [33]. These moths preferred white artificial flowers (feeders) over blue feeders at the lowest light conditions, and this preference changed to their preferred blue color when light levels increased. This may be because these moths use achromatic vision rather than color vision under dim light conditions. Because of the increasing relevance of noise such as photon shot noise under dim light conditions, achromatic vision is likely a good option under such circumstances [34], and diurnal hawkmoths switch between achromatic and chromatic vision based on ambient illumination [35]. In general, it is now widely accepted that detectability changes with light conditions [36]. The consequences of this finding for vision-based features in night-blooming flowers must be explored. For example, it is possible that flowers that bloom at dusk and attract crepuscular pollinators may incorporate different visual features compared to those that bloom at night; differences in the quality and quantity of light available at these times may impose varied selection pressures.

Polarization Patterns at Night

Many diurnal pollinators, such as bees, use polarization patterns in the sky for navigation and orientation [37,38]. However, nocturnal animals could also use polarization patterns. The crepuscular/nocturnal bee *Megalopta genalis* has polarization sensitivity [39], and polarized light, that can be used by this bee during its activity period, is present in the sky for about an hour between astronomical twilight and sunrise/sunset [40]. Night-sky polarization patterns could also be employed by nocturnal pollinators such as bats since the use of polarization cues for navigation has been recently discovered in a single bat species [41]. Dung beetles navigate and orient using night-sky polarization and the Milky Way [2,42]; it is, therefore, possible that nocturnal beetle pollinators may also have the ability to use night-sky polarization. While research using artificial flowers suggests that diurnal bumblebees can learn flower polarization patterns [43], whether nocturnal blooming flowers present such patterns is not known.

Olfaction in the Night

Neural systems are expected to evolve to match sensory challenges. Because light is limiting in the night, it is widely believed that nocturnal animals also use olfactory signals or cues to find their targets [7]. Even when diurnal butterflies specialize on olfaction, their brains reflect an olfactory bias compared to vision [44]. Yet, there is limited work on the comparative olfactory systems of day-active and night-active pollinators even though plants that flower in the night often produce larger quantities of floral scents than closely related species that bloom in the day [45].

How do sensory systems, such as those involved with vision and olfaction, trade off against each other in day-active versus night-active pollinators? A comparison between the diurnal hummingbird hawkmoth *Macroglossum stellatarum* and the nocturnal elephant hawkmoth *Deilephila elpenor* demonstrated an interesting trade-off between vision and olfaction [46]. The lower-order and higher-order olfactory neuropils (dense network of neurons) showed greater investment in the nocturnal species compared to the diurnal one after normalization for volume in relation to size. Furthermore, behavioral tests using multimodal signals (visual plus olfactory) revealed that the nocturnal species paid more attention to olfactory than visual signals in foraging tasks compared to the diurnal species. The brains of diurnal and nocturnal dung beetles also have adaptations that reflect the relative importance of vision versus olfaction in the former relative to the latter [47]. These valuable comparisons between diurnal and nocturnal species indicate that while nocturnal pollinators have many important visual adaptations

to cope with the nocturnal niche, olfaction also plays a very important role in terms of attention to floral signals. This was affirmed in an experiment with *M. sexta* moths. These moths were unresponsive at starlight and crescent moonlight conditions when offered unscented artificial flowers; they could, however, be made to respond by conducting experiments at halfmoon or gibbous moonlight conditions or by adding scent to the flowers [48].

Switching to a Nocturnal Lifestyle

As we have seen, pollinators adapt in various ways to nocturnal life. There are several examples of pollinator taxa within groups that are normally diurnal that have switched to being nocturnal. These include some bees such as *Megalopta* [15] or *Xylocopa tranquebarica* [49] that are able to utilize floral resources under dim light despite having an eye design that is fundamentally suitable for diurnal vision. There is some evidence that diurnal bees have shifted to nocturnal lifestyles to avoid competition [50,51, Somanathan and Borges, unpublished data]. In some nocturnal pollinators, *e.g.,* bats, the whole group has evolved nocturnality, and is therefore likely to have pre-adaptations that enabled the exploitation of the nocturnal pollinator niche. For example, although nectar-feeding bat lineages arose from insectivorous lineages [52,53], nectarivores from these lineages still utilize echolocation as do insectivorous species but integrate this with olfaction to find flower resources in the night [54]. The megachiropteran bats or flying foxes are important nocturnal pollinators and use well developed eyes and color vision in their flower and fruit feeding [55]. There is evidence that mammals as a group may have experienced selection for invasion into dim-light environments in order to occupy empty niches [1]. This switch may have helped mammals such as bats, rodents, and primates to become successful nocturnal pollinators.

FLOWERING IN THE NIGHT: ADJUSTING TO POLLINATORS OR ABIOTIC CONSTRAINTS

Flower Size

Nocturnality in flowering has arisen many times across lineages of flowering plants [7]. Therefore, as in nocturnal pollinators, plants have also had to adjust to the abiotic factors that characterize the night in order to facilitate gamete exchange with other plants. It is not known for sure why some plants within diurnal lineages have undertaken the transition to nocturnal blooming. However, some plants open their stomata only during the night to reduce water loss due to evapotranspiration. This shift in timing of stomatal opening is also often coupled with a special type of photosynthesis referred to as crassulacean

acid metabolism (CAM) and is believed to have evolved during periods when Earth was experiencing hot and dry conditions [56]. Extending this argument further, Borges *et al.* [7] examined the presence of nocturnal pollination in 413 families of angiosperms, and found that nocturnal pollination occurred to a greater extent in families inhabiting drier habitats or microhabitats (*e.g.* upper levels of tree canopies); such families also showed anatomical adaptations, such as laticifers, resin ducts, or special water reservoirs, to increase water-holding capacities. Borges *et al.* [7] therefore hypothesized that opening flowers at night freed plants from excessive loss of water via evapotranspiration from floral surfaces; such escape from water loss possibly enabled plants to provide larger amounts of nectar in their flowers and to evolve larger flowers. Indeed, in plants that open flowers during the day, there is a strong negative relationship between flower size and extent of aridity [57], suggesting that flower size is under negative selection pressure by the impact of water loss through evapotranspiration. In fact, in avocado plants, 13 percent of transpirational water loss is from flowers [58]; also, plants with large inflorescences such as *Agave deserti* require about 18 kg of water during flowering [59]. Therefore, opening flowers at night and removing the constraint on flower or inflorescence size, could have resulted in the co-evolution of much larger-flowered plants with larger pollinators such as bats or large hawkmoths.

Nocturnal blooming flowers or inflorescences can, therefore, afford to be larger than their diurnal counterparts. This remains to be rigorously tested in comparison with related diurnally blooming flowers but appears to be a reasonable expectation. Even if nocturnally blooming flowers are small, owing for example to phylogenetic constraints, it is possible that individual plants could mount a larger overall floral display to make them more detectable at night. This is seen, for example, in *Erica lanuginosa* (Ericaceae), a nocturnally-blooming rodent-pollinated South African plant that bears many small flowers, each of which is adapted to being handled and having nectar removed only by nocturnal rodents [60]. It is also possible that plants have opted to bloom in the night to protect their pollen from heat stress since the maturation of pollen is one of the most temperature-sensitive processes in plants [61].

The presence of pigments such as flavonoids in flowers likely originated as protection against damaging ultraviolet (UV) radiation [62] and could also ameliorate heat stress [63]. If this is the case, then plants that bloom at night no longer run the risk of UV damage to reproductive tissue, and also experience lower heat stress; this may have led to the selection of white, non-pigmented flowers [7]. A by-product of this pale coloration could have been an increase in achromatic contrast in the night thus increasing flower detectability. However, that many

nocturnal animals, including pollinators, retain color vision [14,49,55], suggests that pale coloration in nocturnal flowers is primarily being driven from a plant perspective and that nocturnal color vision in pollinators is being maintained by other factors. However, this hypothesis remains to be rigorously tested.

Floral Scent

Floral scent attracts biotic pollinating agents. A comprehensive meta-analysis demonstrated that insect-pollinated plants produce scents in quantities that are orders of magnitude higher compared to those that are wind-pollinated [64]. Recent evidence that floral scent emission is governed by ABC transporters [65] indicates that scent emission is an energy-demanding process and is not exclusively dictated by passive diffusion through the flower surface. Therefore, it is reasonable to assume that plants emit scents when required. Indeed, many plants demonstrate a clear-cut diurnal rhythm in floral scent emission with nocturnally blooming flowers emitting scent primarily during the night and shutting down emission during the day [45]; however, there are exceptions [66]. Nocturnal flowers are also more strongly scented than diurnal flowers; however, there is as yet no systematic comparison between the scent quantities produced by nocturnal flowers in comparison to related diurnal species although a few cases exist. For example, nocturnal flowering cacti are more strongly scented than those that flower during the day [67]. Also, the flowers of *Clarkia breweri*, which is moth-pollinated at night, produce 8 to 15 times more volatiles [45], and some volatiles are produced at a 200 times faster rate [68], when compared to those of *Clarkia concinna* which is a sympatric day-pollinated species.

Flower Color and Scent Patterns

Many diurnally opening flowers have color patterns that could serve as "nectar guides" to pollinators [69-72]. This is usually a distinctive pattern that could be strongly reflective in the UV and that serves to "guide" the pollinator at close range towards the nectar-containing region of the flower; it may also consist of polarization patterns that pollinators may learn [43]. The UV component of nectar guides in diurnal flowers appears to exploit the UV sensitivity of many insect pollinators [14]. As mentioned earlier, UV-absorbing pigments on flowers may protect diurnally opening flowers from UV damage in areas or altitudes that receive high UV-B irradiance [62]. Visual nectar guides in nocturnal flowers have not yet been documented. Therefore, it is interesting that nocturnal flowers may use olfactory nectar guides to inform pollinators at close range about the location of the rewards [73]. In the nocturnally blooming *Silene latifolia*, male and female flowers emit lilac compounds from the male

and female reproductive organs; such compounds are attractive to the pollinators and serve as guides [73]. As in the use of UV patterns to which insect pollinators may innately respond, some nocturnally blooming flowers employ aromas to which their mammal pollinators have an innate response. For example, the flowers of the nocturnal ground-blooming parasitic plant *Cytinus visseri* (Cytinaceae) produce scents dominated by ketones such as 3-hexanone to which their rodent pollinators are innately attracted [74].

Floral Heat

Some nocturnally pollinated flowers within a few angiosperm families such as Nympheaceae, Araceae, and Arecaceae/Palmae offer heat to pollinators as a reward [7]. In this case, the pollinators are usually scarab beetles [7] that enter the flowers at dusk, mate, and feed within the floral chamber throughout the night, and leave at dawn [75]. Such inflorescences are also large and may use their thermogenicity to volatilize scents that are attractive to beetles. In a comprehensive study of the physiology of scarab beetles that pollinate *Philodendron slimoesense* (Araceae), it was found that these beetles are endothermic (generate their own body heat). However, the beetle's requirements for energy when outside the thermogenic flower are 2 to 5 times higher than when they are within the flower; the heat generated by the flower enables them to save on energy during the night since within the floral chamber the mean temperatures were 3.4 to 5°C above ambient [75]. Such energy savings could be even greater and more critical for the pollinators of some tropical thermogenic flowers that flower at periods when night temperatures drop quite low as in the Brazilian highlands [75,76].

Flower Carbon Dioxide as a Pollinator Cue

Carbon dioxide is another important cue that nocturnal pollinators could use to find suitable floral resources. Insects, and therefore also insect pollinators, have exquisitely sensitive CO_2 detection mechanisms [77]. In some nocturnally blooming flowers such as *Datura*, nectar and CO_2 production are correlated; therefore, CO_2 can serve as a good cue for the amount of available nectar [78]. Moths prefer to feed from artificial flowers that emit high levels of CO_2 comparable to those emitted by newly-opened flowers [79] suggesting that CO_2 could be used by moths as a reliable cue for flowers with high levels of nectar rewards. In *Datura wrightii*, CO_2 levels at anthesis were 200 ppm above ambient levels, which is when nectar levels are also highest; moths use CO_2 as a long-distance attractant similar to floral scent and they can also perceive a change in CO_2 concentration as low as 0.5 ppm [80].

Flower Humidity as a Pollinator Cue

Since humidity tends to be higher in the night compared to the day, could humidity around flowers be used as a cue to signal floral rewards? This is possible and has been discovered in night-blooming flowers such as the desert evening primrose, *Oenothera caespitosa,* which is typically found in drier habitats. In such flowers, humidity was found to peak to about 4 percent above ambient levels around newly-opened flowers that also contained the most nectar rewards [81]; moths were able to discriminate between small differences in humidity when all other floral factors were kept constant, suggesting that humidity sensors in nocturnal insect pollinators could help them make the best decisions about choosing which flowers to probe. Insects have specialized hygrosensitive neurons with dedicated sensilla that also contain temperature-sensitive receptors [82,83] suggesting that temperature and humidity, which are ecologically extremely relevant factors, and often correlated, are sensed conjointly.

Multimodal Signaling

Floral signals are complex and multimodal [84,85] and many pollinators need multimodal signals to complete their behavioral repertoires at flowers [86,87] or even to reach floral targets faster and more efficiently [88]. Nocturnal hawkmoths when tested under simulated moonlight conditions seemed to be respond to changes in both corolla shape and flower size [89]. This is also because nocturnal pollinators, such as hawkmoths, handle some floral shapes better than others [90]. While most research on multimodal signals has been performed using diurnal pollinators, nocturnal flowers also employ multimodal signals. For example, many nocturnal flowers couple strong odor with achromaticity that confers high detectability [76]. In experiments with artificial flowers, nocturnal hawkmoths also relied on tactile cues from grooves on these flowers to correctly orient their proboscis [91]. The nature of such tactile cues in real nocturnal flowers and their impact on pollinators is unknown. A valuable recent review on the biochemical networks governing the expression of multimodal signals (pigments and scents) in flowers provides important leads for research on co-expression of these signals in plants [92].

ARTIFICIAL LIGHT AT NIGHT: CONSEQUENCES FOR POLLINATION IN THE DARK

While the previous sections have examined the challenges that nocturnal pollinators face when dealing with too little light at night, a new evolutionary challenge is for nocturnally-adapted organisms to cope with artificial light at night (ALAN). Recent estimates indicated that,

excluding Antarctica, about 19 percent of Earth's terrestrial surface experiences nocturnal light pollution [93]. ALAN can affect flowering and response to photoperiodism in plants since plant physiology and growth are heavily influenced by light via a range of photoreceptors [94]. Despite investigation of the disruptive effect of night light on the physiology of a few model plant species, there have been very few studies on the effect of ALAN on wild plants [94]. The impact of ALAN on plant reproduction can also manifest through disruptions of plant–animal interactions via effects on pollinators and seed dispersers. There are now many studies indicating that ALAN affects the behavior of moths [95]. ALAN changes the composition of the sex pheromone of the cabbage moth *Mamestra brassicae* [96] and also results in inhibition of diapause and sex-specific changes in life history [97]. Although ALAN has negative effects on the foraging and commuting behavior of bats [98,99], only a few studies have shown this for plant-visiting bat species [100]. While there is variation in the sensitivity of moths to ALAN as determined by differential attraction of moth species to light traps [101], there was reduction in pollen transport by moths at sites with street lighting [102]. Nocturnal pollination and fruit set was reduced by ALAN as reported in a recent study [103]. The evidence is mounting that ALAN needs to be taken very seriously for its impacts on inter-species interactions such as nocturnal pollination.

Given that besides light pollution, the planet is also experiencing the impacts of climate change including increased temperatures [104], it is possible that many wild plants and animals may begin to show shifts towards nocturnal activities to avoid temperature stress. The consequences of these nocturnal shifts for life on Earth must be examined.

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