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Direct benefits and indirect costs of warm temperatures for high-elevation populations of a solitary bee

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Abstract. Warm temperatures are required for insect flight. Consequently, warming could benefit many high-latitude and high-altitude insects by increasing opportunities for foraging or oviposition. However, warming can also alter species interactions, including interactions with natural enemies, making the net effect of rising temperatures on population growth rate difficult to predict. We investigated the temperature-dependence of nesting activity and lifetime reproductive output over 3 yr in subalpine populations of a pollen-specialist bee, *Osmia iridis*. Rates of nest provisioning increased with ambient temperatures and with availability of floral resources, as expected. However, warmer conditions did not increase lifetime reproductive output. Lifetime offspring production was best explained by rates of brood parasitism (by the wasp *Sapyga*), which increased with temperature. Direct observations of bee and parasite activity suggest that although activity of both species is favored by warmer temperatures, bees can be active at lower ambient temperatures, while wasps are active only at higher temperatures. Thus, direct benefits to the bees of warmer temperatures were nullified by indirect costs associated with increased parasite activity. To date, most studies of climate-change effects on pollinators have focused on changing interactions between pollinators and their floral host-plants (i.e., bottom-up processes). Our results suggest that natural enemies (i.e., top-down forces) can play a key role in pollinator population regulation and should not be overlooked in forecasts of pollinator responses to climate change.

Key words: brood parasites; climate change; floral resources; Megachilidae; oligolecty; Rocky Mountains; Sapygidae; solitary bees; temperature; top-down control.

Introduction

All else being equal, we should expect ectotherms inhabiting cool climates to benefit from rising temperatures, because they currently spend most of their lives below their thermal optima (Deutsch et al. 2008). For winged insects, the need for warmth is acute because foraging and reproduction often require flight, and flight requires elevated thoracic temperatures (Dudley 2002). Indeed, there is strong evidence from several butterfly species that reproductive output can be limited by the availability of good weather for flight and oviposition (Kingsolver 1989). Although many bee species (including mason bees, *Osmia* spp.) are partially endothermic (Stone and Willmer 1989), they are also affected by ambient temperatures: warmer temperatures (up to a species' upper critical temperature) lower the energetic costs of flight and favor activity (Willmer 1983, Herrera 1995, Cameron et al. 1996, Vicens and Bosch 2000). If reproductive output in bees is limited by foraging opportunities—as seems likely, given their need to provision food for their offspring—we might expect warmer temperatures to increase population growth.

However, factors other than active-season temperatures—such as the abundance of food or the activity of natural enemies—can limit ectotherm populations, and these factors may themselves be affected by temperature. Indeed, virtually all populations are influenced by both "top-down" and "bottom-up" forces. In the case of bees, populations may be limited "bottom-up" by the availability of floral resources (reviewed by Roulston and Goodell 2011). This possibility underlies concerns that shifts in flowering phenology relative to the timing of bee nesting could negatively affect bee populations (Straka and Starzomski 2014). If increases in temperature were to alter flower or nectar production, this too could affect bee populations (Scaven and Rafferty 2013). The role of native natural enemies (top-down factors) in regulating solitary bee populations tends to be overlooked relative to that of floral resources (but see Steffan-Dewenter and Schiele 2008, Rodríguez-Gironés 2012). Yet solitary bees are attacked by a range of parasitoids and brood parasites, and these can be important agents of mortality (Krombein 1967, Torchio 1979, Seidelmann 1999, Münster-Swendsen and Calabuig 2000). These parasites could benefit from warming temperatures for the same reasons as their hosts. In fact, in some insect host-parasite

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systems, parasites are more strongly benefited by warmer temperatures than their hosts (Virtanen and Neuvonen 1999). Thus, it is unclear whether the net effect of warming will be positive, negative, or null, even for bee populations that are currently limited by cool temperatures.

In this study, we ask whether high-elevation populations of a floral-specialist solitary bee, *Osmia iridis*, are limited by temperature, floral resources, or parasites—or by some combination of these factors. To answer this question, we observed nesting progress and reproductive output of individually marked bees over 3 yr and several study sites, in a region where summer temperatures have risen ~0.5°C/decade in recent decades (Aldridge et al. 2011, Kingsolver and Buckley 2015) and are expected to rise another 2–4°C by 2100 (IPCC 2013). We also collected data on local temperatures, flower density, levels of parasitism, and the temperature-dependence of bee and parasite activity. Using these data, we determine if warming temperatures are likely to be a net cost or benefit to these bee populations.

Methods

Study system

Osmia (Hapsidosmia) iridis Cockerell & Titus is a solitary mason bee that nests in existing aboveground cavities in woody material (Fig. 1a). It occurs throughout the western USA (Rightmyer et al. 2013) and is a common occupant of experimental nesting blocks ("trapnests") in our study area (Forrest and Thomson 2011). Nests consist of a linear series of brood cells, each provisioned with a mass of pollen and nectar and containing a single egg. Cells are separated by mud partitions. Bees construct one nest at a time but may complete several nests over a lifetime. We confirmed our field identifications of *O. iridis* by rearing offspring of our focal bees and collecting these as vouchers (to be deposited in the Canadian National Collection in Ottawa, Canada). However, because *O. iridis* are typically semivoltine in our study area (i.e., taking 2 yr to complete a

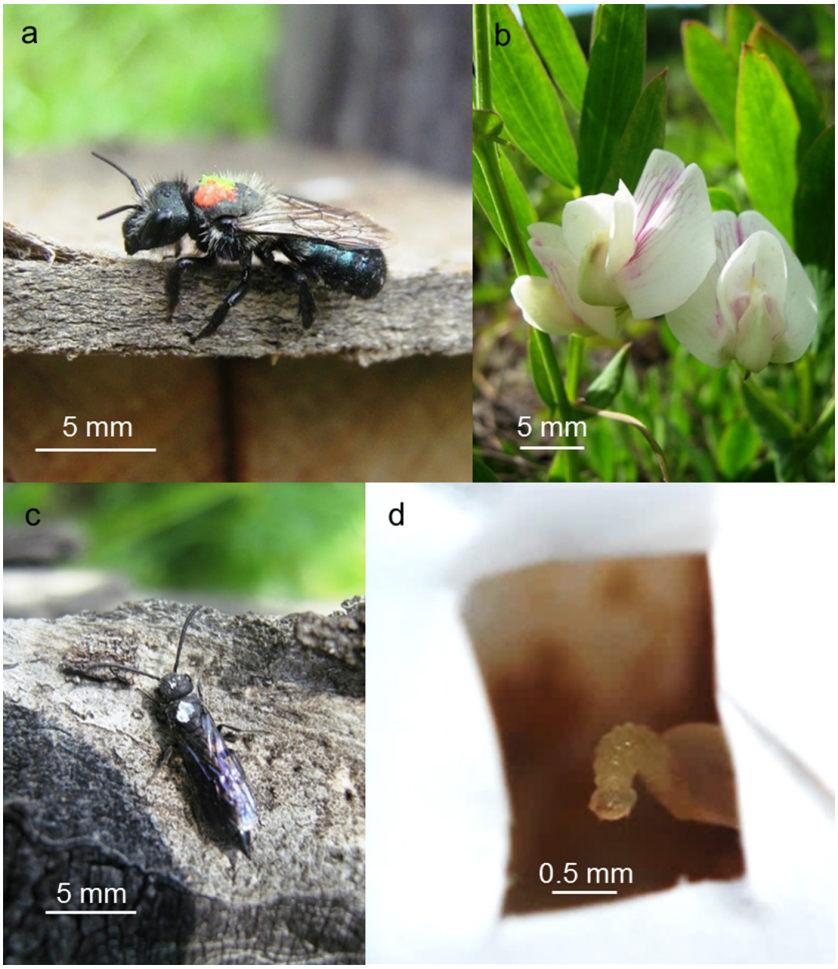


Fig. 1. Photos of study system. (a) Marked female *Osmia iridis* on nesting block. (b) Three *Lathyrus lanszwertii* flowers. (c) Marked female *Sapyga* sp. (d) *Sapyga* larva (center) feeding on an egg of *O. iridis* (below, at right), seen through a window cut in the paper straw housing the nest.

generation), vouchers are available only for bees that constructed nests in 2013 and 2014. Nests built in 2015 are assumed to have been constructed by *O. iridis* if they contained >95% Fabeae pollen by volume (see *Nest dissection*, below). Based on examination of pollen contents from 44 confirmed *O. iridis* nests, all of which contained >95% Fabeae pollen (J. Forrest, *unpublished data*), this species is a pollen-specialist on plants of the legume tribe Fabeae, which in our area is represented by *Lathyrus lanszwertii* Kellogg (henceforth "*Lathyrus*"; Fig. 1b) and *Vicia americana* Muhl. ex Willd. ("*Vicia*"; pollen of the two species is indistinguishable). Both plant species are herbaceous perennial vines that are widespread in the western USA. No other trap-nesting *Osmia* species in our study area is a specialist on Fabeae.

The most abundant brood parasite (kleptoparasite) of *O. iridis* in our study area is the wasp *Sapyga pumila* Cresson (Sapygidae; Fig. 1c), which oviposits through recently completed nest partitions of several cavity-nesting *Osmia* species (Spear et al. 2016). Only the most recently completed nest cell—the one nearest the nest entrance—is vulnerable to this parasite. The sapygid egg hatches quickly and the young larva immediately kills the egg of the host bee (Fig. 1d) before consuming the pollen provision (Torchio 1972).

We studied *O. iridis* populations using artificial nesting structures established at sites along an elevational gradient. We used the seasonal, among-year, and among-site (elevational) variation in temperature and floral abundance to characterize the dependence of bee nesting rates and parasitism rates on temperature and floral resources. We used among-site and among-year variation in temperature, floral abundance, and parasitism rate to determine the influence of these factors on bee per capita (annual) reproductive output. Finally, we observed insect activity in one summer at a subset of sites to characterize the temperature-dependence of bee and parasite activity. These methods are described in detail herein.

Study sites

Study sites were located at the margins of subalpine meadows around the Rocky Mountain Biological Laboratory (Crested Butte, Colorado, USA). Meadows were dominated by perennial forbs and bordered by aspen (*Populus tremuloides*) or spruce-fir forest (*Abies lasiocarpa* and *Picea engelmannii*). Climate in this area is characterized by heavy winter snowfall, which accounts for most of the annual precipitation; drought in early summer (June); and late-summer monsoons. We established nesting blocks at five sites in May–June 2013 and an additional two sites in May–June 2014 (Appendix S1). Each nesting block was roofed with hardboard and consisted of sections of untreated softwood lumber with 10 tunnels drilled through. Tunnels were ~14 cm deep and 6.4–9.5 mm diameter; each was lined with a translucent paper straw of appropriate size (Custom Paper Tubes, Cleveland, Ohio, USA). At six of the sites, 14 blocks were attached to trees (usually standing dead aspens) at 0.3–1 m above ground level. For reasons related to a separate study, we set up 24 nesting blocks at the seventh site (RP), at 0.5–1.5 m above the ground. At each site, we attached a HOBO pendant data-logger (Onset Computer, Bourne, Massachusetts, USA; accuracy ±0.5°C) to the underside of one centrally located nesting block to record temperature hourly.

Trap-nest monitoring

In 2013, sites were checked weekly until the first bees were observed nesting in the nest blocks; we then switched to more frequent checks (every 3–4 d) until the end of *O. iridis* nesting in mid-August. In 2014 and 2015, all sites were initially checked every 3–4 days, but monitoring tapered off to every 5–8 days late in the season (late July–August) when bee activity slowed.

During each site visit, we checked all nest holes for occupancy and nesting progress by temporarily extracting the straw liners. (A nest consists of the contents of a single straw.) These brief checks typically occurred while the bee was away from the nest and are unlikely to have affected nesting behavior. The outer surface of the straw was marked at the location of the outermost pollen provision or completed cell wall, and the number of pollen-containing brood cells constructed since the last date was estimated to the nearest 0.5 cell in 2013, and to the nearest 0.1 cell in 2014–2015 (e.g., a seemingly complete provision without an egg would be 0.8; pollen from a single foraging trip would be 0.1). The date of construction of each cell was estimated by interpolation.

To track individual bees, we applied a paint mark to the mesosoma of each nesting *Osmia* (Testors hobbyist's paint; Vernon Hills, Illinois, USA). Bees typically flew away after being marked and then returned to nesting. We attempted to determine the individual occupant of each nest, but some nests were completed without our ever seeing the occupant. These nests were excluded from calculations of individual-level nest progress or reproductive rate, but were included in site-level calculations of parasitism rate (see *Per capita reproductive output*, below).

Nest dissection

At each site visit, we inspected recently completed nest cells for eggs of brood parasites; a few nests were damaged or could not be extracted for dissection. We cut small windows in the paper straws adjacent to each pollen provision and scored each nest cell as parasitized (Fig. 1d) or unparasitized. Parasite presence was later confirmed based on emerged adults or by examination of cocoons (2013–2014), or by monitoring larval development (2015). To determine which pollen types were present in each nest, we sampled the pollen provision from at least one cell per nest, as described by Spear et al. (2016).

Floral resource availability

At each site visit during the bee nesting period in 2013, and at each site visit in 2014–2015, we estimated density of the floral host-plants (*Lathyrus* and *Vicia*). We did this by walking in progressively larger circles from a central point, counting open flowers until we reached a total of 100 flowers of each species or a distance of 100 m (straight-line distance) from the center, whichever came first. We counted flowers only within a 100 m radius because solitary bees of similar size to *O. iridis* forage predominantly within 100 m of the nest (Zurbuchen et al. 2010) and surveying larger distances would have been impractical. We then measured the distance (r) to the 100th flower and calculated density of that species as $100/\pi r^2$. (If we found fewer than 100 flowers—a situation in which the maximum actual density would be $0.003/m^2$—density was recorded as 0.) Like other sampling methods, this approach is imperfect: it can overestimate floral density when r is low, and it underestimates density when flowers are scarce. However, we chose it because (1) it adjusts sampling area to the density of each species, (2) it allows us to sample patches that might be missed by plot- or transect-based methods, and (3) it seems a reasonable approximation of how a bee (a central-place forager) experiences floral density.

We verified that flower density was a reasonable proxy for floral resource availability by sampling the pollen in *Lathyrus* flowers (*Lathyrus* was far more abundant than *Vicia* at our study sites) throughout summer 2014 at one site (Appendix S2). Because oligolectic bees are specialists for pollen but not for nectar (Wcislo and Cane 1996), we assumed that pollen was more likely to be the limiting floral resource and did not attempt to measure nectar availability. Pollen per flower varied by 31–56% among weeks (depending on the method of measurement) and 22–35% over the course of a day, but did not vary systematically over the season (Appendix S2); in contrast, floral density varied by 145% over the flowering period at the same site.

To estimate floral density on each day of the season (including days on which we did not sample), we fitted a cubic spline with the R package "stats" (R Core Team 2015) to the observed floral densities (Appendix S3). We used these interpolated floral-density values for all analyses.

Temperature-dependence of bee and parasite activity

To directly quantify the temperature-dependence of host bee and brood-parasite activity, we observed individually marked bees and wasps during half-hour intervals between 0900 and 1600 h at four sites on 11 d (total) in 2015. Wasps were marked as described earlier for bees. Observations were conducted by one observer (SC) at 1–2 focal nest blocks at a time; at the end of each 30-min interval, the observer switched to a different block (or pair of blocks). We recorded whether any sapygid wasps were present at the nest block over the 30-min interval. For each individually marked *O. iridis* bee, we recorded the amount of time spent away from the nest and the amount of time spent in the nest. We assume that most time away from the nest was spent foraging, but it may include periods of rest as well. For individually marked wasps, we recorded the amount of time spent flying in the vicinity of the nest block (within ~30 cm) and the amount of time spent sitting on or near (within 30 cm of) the nest block. At the beginning and end of each observation period, we recorded air temperatures adjacent to the nest block using an unshaded Kestrel 2000 weather meter (Kestrel Meters, Birmingham, Michigan, USA), to capture temperature as experienced by the insects. For subsequent analysis, we used the average of the initial and final temperature measurements as the mean temperature for each 30-minute interval.

Data analysis

Rate of nesting progress.—We analyzed rate of nesting progress as a function of ambient temperature and host floral density using linear mixed models with the R package "lme4" (Bates et al. 2015). Here, the units of replication were observations of individual nests, made every 3–8 d. The response variable was the number of cells (or fractions of cells) completed per day by each individual bee; this was square-root-transformed to improve normality. Our temperature metric was the number of daily hours >16°C, as recorded by the HOBO logger at each site, averaged over the time since the previous observation. (We tried all temperature thresholds between 13° and 20°C in 1° increments and chose 16°C based on Akaike's information criterion [AIC] values.) Floral density was averaged over the relevant time interval and square-root-transformed to minimize the influence of a few extreme values. We included year (categorical) and day of year as covariates in the model and included individual bee, nested within site, as a random factor. We used a random-intercepts model (i.e., we did not allow slopes to vary among bees or sites; Hox and Roberts 2011) because we did not have sufficient observations of each bee to fit random slopes. We used the package "lmerTest" (Kuznetsova et al. 2015), which uses Satterthwaite-approximated degrees of freedom, to evaluate significance of fixed factors. We used the "rsquared.GLMM" command in package MuMIn (Barton 2016) to obtain marginal "R^2" values from mixed models—i.e., measures of the proportions of variance explained by the fixed factors in the model. Although there were correlations among predictor variables (in particular, floral density and day of year were negatively correlated; $r = -0.64$, $N = 485$ observations), variance inflation factors were modest (maximum = 2.6), suggesting that multicollinearity was not a major problem.

Per capita reproductive output.—For each site in each year, we calculated the number of unparasitized nest

cells produced by each marked *O. iridis* individual (i.e., the subset of that bee's nest cells that did not contain parasite eggs) as our measure of potential per capita reproductive output, "R_0". We modeled R_0 (square-root-transformed, using linear mixed-effects models) as a function of temperature, floral density (square-root-transformed), parasitism rate at the site, and year; site was included as a random factor. Here, the temperature metric was the mean temperature in June–July (obtained from the HOBO loggers); this gave a slightly lower AIC (ΔAIC = 0.3–1.5) than temperature predictors based on mean numbers of daily hours above various threshold temperatures. For floral density, we used the maximum density of Fabeae flowers observed at the site over the course of the *O. iridis* nesting period at that site; this gave better model fits than other metrics (e.g., summed density of host flowers over the entire summer). Parasitism rate was the proportion of all *O. iridis* cells at a site that were parasitized. Correlations among site-level variables are presented in Appendix S4.

We ran the same analysis for the total number of nest cells per marked *O. iridis* individual at each site—i.e., including parasitized cells in the total—to assess how offspring production varied as a function of floral host density, temperature, and parasite attack rate, independent of the direct effect of parasites on offspring survival.

Temperature-dependence of parasitism and floral density.—We tested for a relationship between temperature and the probability of an individual nest cell being parasitized using a generalized linear mixed model (GLMM) with binomial error distribution. The predictor variable of interest was the number of hours >16°C on the estimated day on which that cell was constructed (again, this gave a lower AIC than other temperature thresholds tested). Year and day of year of nest-cell construction (rescaled to mean = 0 and SD = 1) were included as additional fixed factors, and nest identity, nested within site, was included as a random term. This analysis included all brood cells completed by *O. iridis*, including unmarked individuals.

We tested for a relationship between summer temperatures and floral density at the site level using a linear mixed model of the summed density of Fabeae flowers (integrated over the entire season and square-root transformed; a model using maximum floral density gave qualitatively identical results). Predictor variables were mean June–July temperature, year (categorical, fixed), and site (random).

Temperature-dependence of bee and parasite activity.—We modeled sapygid presence at nest blocks during 30-min observation periods (a binary variable) as a function of ambient air temperature using a binomial GLMM. Block identity (nested within site) and day of observation were included as random factors. We used models of the same structure to analyze the activity of individual *O. iridis* bees and *Sapyga* wasps: For bees, the response variable was the proportion of time spent away from the nest during 30-min observation periods; for wasps, the response variable was the amount of time spent flying relative to the total time present at the nest.

For all models, we checked diagnostic plots to verify that model assumptions were met.

Results

In all, 109 marked *Osmia iridis* bees constructed 185 nests and 924 nest cells at our study sites. An additional 84 *O. iridis* nests (149 cells) were constructed by unknown (unobserved) individuals.

Over the 3 yr of study, bee nesting progressed more rapidly on warmer days ($F_{1,425} = 89.2$, $P < 0.0001$, Fig. 2a). Rate of nesting progress also increased with floral density ($F_{1,384} = 8.2$, $P = 0.0044$, Fig. 2b) and decreased over the course of the season (−0.0075 nest cells per day; $F_{1,401} = 14.7$, $P = 0.0001$), but did not differ significantly among years ($F_{2,78} = 1.7$, $P = 0.20$). Total floral density (summed over the season) at a site in a given year was unrelated to mean June–July temperatures ($F_{1,5.3} = 0.14$, $P = 0.72$, $N = 19$ observations).

At the site level, mean per capita reproductive output ("R_0", the number of unparasitized offspring per mother) varied from 0.9 to 25.5. R_0 of individual bees varied significantly among years but was not significantly associated with mean summer temperature or maximum floral density at the site (Table 1, Fig. 3a–b). However, R_0 declined strongly as the site-level parasitism rate increased (Table 1, Fig. 3c). Parasitism rate was also the strongest predictor of per capita nest-cell production; i.e., the number of cells produced per marked bee declined with increasing parasitism rate, irrespective of whether these cells were ultimately parasitized (Table 1, Fig. 3d). Year was also a significant predictor of total nest-cell production, but temperature was not; floral density had a marginal positive effect (Table 1).

When we re-ran these analyses using parasitism rates calculated only from unidentified bees—i.e., from a smaller but independent set of nest cells from the ones used to calculate R_0—only parasitism rate and year remained significant predictors of R_0 (parasitism: $F_{1,19} = 7.8$, $P = 0.012$; year: $F_{2,16} = 4.8$, $P = 0.024$) and of total nest-cell production (parasitism: $F_{1,28} = 4.6$, $P = 0.040$; year: $F_{2,23} = 4.8$, $P = 0.018$).

Overall, 21.7% of nest cells were parasitized. Most parasites (89%, based on 2013–2014 data for which parasite identities are confirmed) were the brood-parasitic wasp *Sapyga*; the remainder were parasitoid wasps (Ichneumonidae and Pteromalidae) and beetles (*Trichodes*; Cleridae). Sapygid presence around nest boxes was more frequent at higher ambient temperatures ($\beta = 0.32$, $z = 4.2$, $P < 0.0001$, $N = 150$ observations; Fig. 4a). While both bees and wasps tended to be more active with increasing ambient temperature (bees: $\beta = 0.46$, $z = 4.0$, $P < 0.0001$, $N = 82$ observations; wasps:

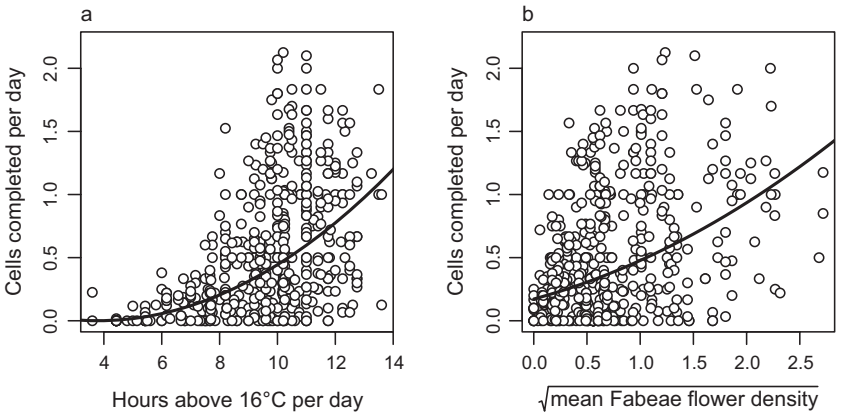


FIG. 2. Rate of nesting progress (number of nest cells per day) vs. (a) temperature and (b) density of host-plant flowers for 109 individually marked *Osmia iridis* bees at seven study sites across 3 yr. $N = 487$ observations. Lines are linear mixed-effects model fits of the form √Cells per day ~ Predictor + (1|Site/Individual), where $y \sim x$ indicates that y is a function of x, $(1|X)$ indicates that X is a random factor, and X/Y indicates that Y is nested within X. Lines are plotted as Cells per day = $(a + b \cdot \text{Predictor})^2$, where a and b are, respectively, the estimated intercept and slope. Marginal R^2 of the fitted model is 0.29 in (a) and 0.14 in (b). See Appendix S5 for a version of this figure with points color- and symbol-coded by site and year.

$\beta = 0.41$, $z = 1.4$, $P = 0.15$, $N = 29$ observations), wasps appear to require warmer temperatures than bees for flight (Fig. 4b; note, however, that intercept parameters do not differ significantly between bees and wasps). As would be expected from these results, the probability of a nest cell being parasitized increased weakly but significantly with temperature on the estimated date of cell construction ($\beta = 0.14$, $z = 2.4$, $P = 0.017$, $N = 996$ cells; Fig. 5). Neither year nor day of year of cell construction was a significant predictor of parasitism ($|z| < 0.8$, $P > 0.4$).

DISCUSSION

Warm daytime temperatures in summer directly benefit *Osmia iridis*. Activity levels and rate of nest construction—and therefore of offspring production—increased strongly with temperature. Based solely on foraging rates, therefore, we would expect warmer temperatures to benefit *O. iridis* populations in our study area. However, there was no detectable effect of summer temperature on annual reproductive output, because warm temperatures also benefit the bees' primary parasite, *Sapyga*. Warm temperatures do not appear detrimental to the floral host-plant, *Lathyrus lanszwertii*; if anything, the relationship between summer temperatures and floral density was positive (though non-significant) across our study sites and years. In our system, indirect negative impacts of warm temperatures are likely "top-down" (driven by positive effects on the bees' natural enemies) rather than "bottom-up" (driven by negative effects on their food supply).

Temperature, parasites, and bee reproductive output

Incidence of parasitism was the strongest predictor of per capita reproductive output in our bee populations, suggesting that parasites play an important role in population regulation. Although some introduced predators consume native bees (Abe et al. 2008, Wilson and Holway

TABLE 1. Linear mixed models of bee reproductive output as a function of site-level predictor variables ("site" included as a random term in models).

Response	Predictor	Estimate	F	df	P
R_0	Mean Jun–Jul temperature	0.02	0.004	1, 33.8	0.95
	Sqrt(max. floral-host density)	0.30	2.31	1, 45.0	0.14
	Parasitism rate	−4.11	35.4	1, 14.6	<0.0001
	Year		4.45	2, 69.3	0.015
Total nest cells per capita	Mean Jun–Jul temperature	−0.10	0.15	1, 41.6	0.70
	Sqrt(max. floral-host density)	0.35	3.72	1, 62.6	0.058
	Parasitism rate	−2.87	20.0	1, 21.7	0.0002
	Year		4.93	2, 73.1	0.010

Notes: "R_0" is the number of unparasitized brood cells per bee; "total nest cells per capita" includes parasitized cells. Both response variables were square-root-transformed for analysis. Parameter estimates are listed for continuous predictors. $N = 104$ bees over 17 site–years.

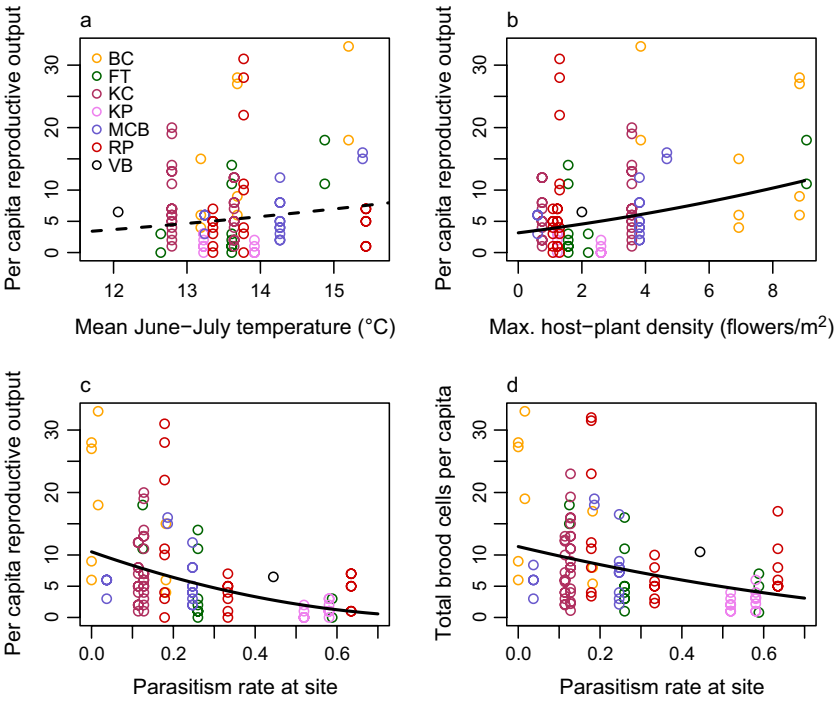


FIG. 3. Bee per capita offspring production vs. (a) temperature, (b) density of host-plant flowers, and (c–d) parasitism rate, measured as the proportion of all *Osmia iridis* cells at a site that were parasitized, for seven study sites across 3 yr (N = 108 bees and 17 site-year combinations). Panels (a–c) show numbers of unparasitized nest cells per bee as the response variable ("R_0"); (d) shows the total number of nest cells per bee. Colors represent different study sites; points represent individual bees. Regression lines are linear mixed-effects model fits of the form √Response ~ Predictor + (1|Site); marginal R^2 values: (a) 0.02, (b) 0.08, (c) 0.25, (d) 0.15. Lines are solid if the effect of the predictor is significant ($P < 0.05$) in these univariate regressions and dashed otherwise.

2010), there has been little evidence to date of natural enemies regulating populations of wild bees (Roulston and Goodell 2011). The lack of evidence of demographic impacts in wild populations is surprising in light of the widely recognized role of parasites in honey bee declines (Vanengelsdorp and Meixner 2010) and the numerous studies of predator and pathogen impacts on bee foraging behavior and pollination (Meehan et al. 2005, Robertson and Klemash Maguire 2005, Gillespie and Adler 2013). Our study is correlative, so we cannot be certain that the negative association between parasitism rate and bee offspring production is a causal one. (Experimental manipulation of parasite presence is not feasible at a scale that would allow normal foraging by bees or their parasites.) However, a causal relationship is likely, both because brood parasites kill bee eggs and because parasite attack seemingly causes host bees to prematurely seal nests (A. Groulx and J. Forrest, *unpublished data*). Termination of parasitized nests would further reduce bees' rates of offspring production, owing to the fixed costs of sealing a nest and locating a new nesting site. Premature nest termination may explain why numbers of both unparasitized brood cells (Fig. 3c) and total brood cells per capita (Fig. 3d) are reduced in heavily parasitized sites. We do not find evidence to support an alternative explanation, namely that bees spend more time "guarding" nests, rather than foraging, when parasites are present. In fact, bees spend a greater proportion of their time away from the nest when parasites are present (binomial GLMM, $\beta = 2.3$, $P = 0.013$, $N = 82$ observations)—presumably because activity of both bees and wasps is favored by higher temperatures (the association of bee activity with wasp presence vanishes when ambient temperature is included in the model; $\beta = 0.05$, $P = 0.96$). Longer-term monitoring of bee population sizes in relation to previous years' parasitism rates will be necessary to better evaluate the demographic impacts of the parasites. Nevertheless, our results suggest that natural enemies must be considered in forecasts of pollinator responses to environmental change.

In principle, bees could be more susceptible to parasite attack when floral resources are scarce, because resource scarcity should force bees to spend more time away from the nest, foraging (Goodell 2003). In our study area, sites with low floral density tended to have higher parasitism rates, but the association was not significant (logistic mixed-effects regression of parasitism rates vs. maximum floral-host density and year; $z = -1.6$, $P = 0.11$, $N = 104$ bees). While floral scarcity might make bees more vulnerable to parasite attack, sites with few flowers may also support insufficient bee production to maintain parasite populations.

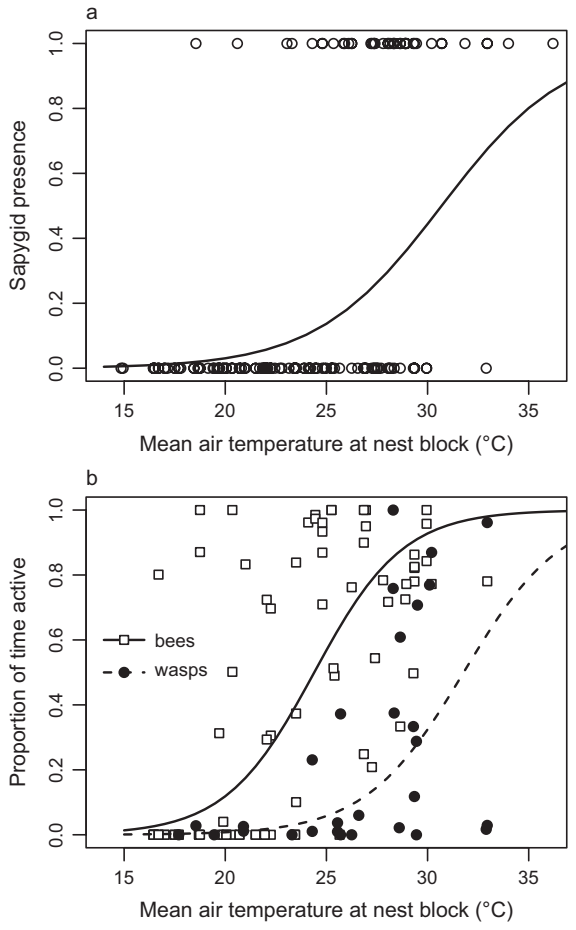


FIG. 4. (a) Brood-parasitic sapygid wasp presence at nest blocks vs. ambient temperature in 30-min intervals. Line is a logistic regression fit of the form Sapygids present? ~ Temperature + (1|Site/Block) + (1|Day of year). N = 150 observations; marginal R^2 = 0.28. (b) Proportion of time in which individual bees (open squares) and brood-parasitic wasps (filled circles) were active (bees: away from the nest; wasps: flying), vs. temperature in a 30-min interval. Lines are logistic regressions of the form Proportion of time active ~ Temperature + (1|Site/Individual ID) + (1|Day of year); models were fitted to each species separately. Bees: N = 82 observations, marginal R^2 = 0.46; wasps: 29 observations, marginal R^2 = 0.34.

Temperature and nesting activity

The climate in our high-elevation study area apparently constrains foraging opportunities for *O. iridis*: bees made little nesting progress if they experienced fewer than 8 daily hours >16°C, and the relationship between nesting rate and temperature does not level off over the range of observed temperatures. The number of days with at least 8 h above 16°C ranges from a maximum of 78–84 at our lowest-elevation site (BC; data from 2013 to 2014) to a minimum of 21–23 at our highest-elevation site (VB). The short window for nesting at the latter site may partially explain why *O. iridis* was scarce at that site (a single nesting female over the 3 yr of study, despite abundant *Lathyrus* flowers).

Bees in the genus *Osmia* are capable of endothermic heat production (Stone and Willmer 1989) and, at lower elevations, are active in the cool conditions of early spring (Krombein 1967). *Osmia* spp. have been reported foraging at air temperatures as low as 10–12°C (Vicens and Bosch 2000, Bosch and Kemp 2001). A threshold of 16°C for activity—as inferred from our results—therefore seems high. However, air temperatures are not necessarily the temperatures experienced by insects. Radiant heat is often a primary determinant of insect activity (Willmer 1983, Herrera 1995, Vicens and Bosch 2000), particularly at high elevations (Kingsolver 1983, Corbet and Huang 2016). Because our dataloggers were not completely sheltered from solar radiation, our measured temperatures include a contribution from radiant heat—and should be more representative than air temperatures of the temperatures experienced by the bees. Indeed, daily maximum temperatures recorded by dataloggers at our BC site are considerably higher (by 5.5°C, on average) than those recorded at a nearby (~5 km distant, 30 m lower elevation) weather station (http://www.ncdc.noaa.gov/). A measurement of 16°C at our sites may therefore correspond to an air temperature of only 10.5°C, in line with previous estimates of threshold temperatures for *Osmia* activity.

In addition to temperature, local floral density and day of year were also significant predictors of rates of nesting progress. The observed seasonal decline in nesting rate may reflect an age-related slowing of bee activity or egg production. The positive effect of floral abundance on nesting progress is unsurprising; previous studies, too, have found strong associations between floral resource

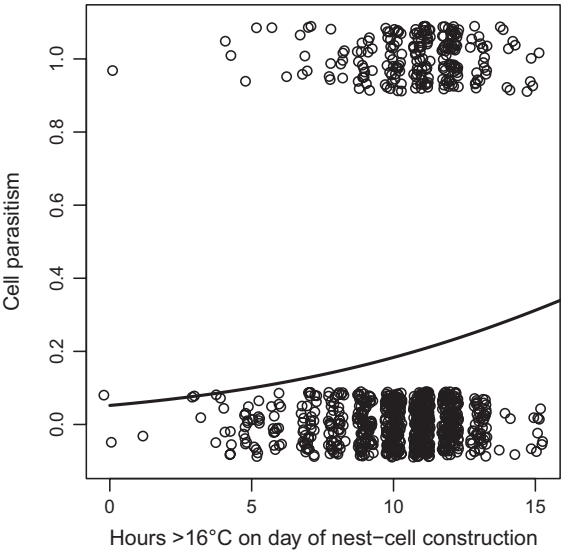


FIG. 5. Probability of a nest cell being parasitized vs. temperature on the day that cell was constructed. Points have been jittered for clarity. Line is a logistic regression fit to the formula Cell parasitized? ~ Temperature + as. factor (Year) + Day of year + (1|Site/Nest). Total N = 996 nest cells; marginal R^2 = 0.02.

availability and population size or foraging rates of oligolectic bees (reviewed by Roulston and Goodell 2011, but see Franzén and Nilsson 2013). Based on the fact that temperature was a stronger predictor than floral density of nesting progress by our bees, it is tempting to conclude that, within the observed range of temperatures and floral densities, bee foraging is more strongly limited by temperature than by floral resources. However, we are cautious about making this inference because we were able to optimize the temperature fits in our models (by testing multiple temperature thresholds) in a way that we could not for floral density. It is safer to conclude that nesting progress is jointly limited by flowers and by temperature.

Implications for a warming climate

How will bees and their brood parasites be affected by future warming? Our observations of insect activity suggest that *O. iridis* and *Sapyga* are similarly responsive to temperature variation; however, the wasps seem to have a higher temperature threshold for activity (Fig. 4b). While our sample size for wasps is small, previous studies have also found that kleptoparasites were more dependent than their hosts on warm external temperatures, being active only when ambient temperatures exceeded 23°C (Straka and Bogusch 2007, Rozen et al. 2009). Kleptoparasitic bees tend to have lower rates of endothermic warming than similar-sized non-parasitic bees (Stone and Willmer 1989). Furthermore, female *O. iridis* are larger and more robust than female *Sapyga* and should therefore better retain body heat. Together, these findings suggest that warm daytime temperatures could benefit brood parasites more than they benefit the host bees.

Several studies in other systems have also concluded that higher trophic levels benefit more than lower trophic levels from warming, such that warming increases top-down control (Barton et al. 2009, O'Connor et al. 2009, Hoekman 2010, Frenken et al. 2016). Similar to our study, Virtanen and Neuvonen (1999) observed higher incidence of parasitism in *Epirrita autumnata* caterpillars at warm sites, and inferred that parasitoid activity was favored by high temperatures. van Nouhuys and Lei (2004) also found that *Cotesia* parasitoids benefited more from warm conditions than their caterpillar (*Melitaea cinxia*) hosts, in that case because of differences in the temperature sensitivity of development rates. However, there are also counterexamples, in which warm conditions disproportionately favor prey over parasites and predators—for example, by allowing prey to more quickly reach a "safe" developmental stage (Dale and Frank 2014, Meineke et al. 2014, Culler et al. 2015). Although there have been attempts to draw general conclusions about which trophic levels will benefit most from warming (Voigt et al. 2003, Berggren et al. 2009), the reality seems to be system-dependent.

Temperature could also influence our study populations by changing rates of development, an aspect we have not addressed here. Indeed, *O. iridis* in our study area is predominantly semivoltine (Forrest and Thomson 2011), and warmer summers may allow bees to complete development in a single year, potentially almost doubling population growth rates. However, *Sapyga* in our area are also typically semivoltine, so again any benefits of warming to bees could be negated by benefits to their parasites. In addition, whether climate change in fact causes bees and their parasites to experience warmer temperatures will depend not only on changing temperatures but also on possible changes in cloud cover, bee phenology, and nest-site selection.

Conclusions

Investigations of pollinator responses to climate change typically focus on how warming may change the temporal availability of flowers (Hegland et al. 2009, Burkle et al. 2013, Pyke et al. 2016) or the production of floral resources (Scaven and Rafferty 2013)—that is, changes in the bottom-up influences on pollinator populations. Yet top-down forces are equally likely to be altered by climate change, and, as our results illustrate, these can also be critical in understanding outcomes for pollinator populations. In general, accurately forecasting the impacts of future warming will require that we consider changes in all the factors that play important roles in population regulation.

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