THE POTENTIAL FOR PHENOLOGICAL MISMATCH BETWEEN *LATHYRUS LITTORALIS* AND *HABROPODA MISERABILIS* WITH CLIMATE WARMING

By

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**ABSTRACT**

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**Premise of research.** Climate change may alter the timing of flowering and pollinator activity to differing degrees, resulting in phenological mismatches between mutualist partners. Assessing the potential for such mismatches requires an understanding of the environmental factors that cue flowering and pollinator activity.

**Methodology.** I characterized the current phenological overlap of the silky beach pea (*Lathyrus littoralis* [Fabaceae]) and one of its main pollinators, the solitary ground-nesting silver bee (*Habropoda miserabilis* [Apidae]), in coastal northwestern California. I then examined associations of abiotic variables in the environment with the spatial variation observed in the phenology of the two species. Based on the relationship of phenology dates with temperature accumulation, I projected changes in phenological overlap with climate warming. Bagging experiments and field observations were used to examine the mutual dependence of the beach pea and silver bee at my study sites. To my knowledge, this study presents the first attempt to characterize the phenology cuing of a solitary ground-nesting bee under field conditions.

**Pivotal results.** Degree-days above freezing best predicted flowering and bee activity, although an interaction of temperature with moisture influenced the onset of flowering as well. Projections based on climate warming predictions suggest that bee emergence will
advance more rapidly than flowering with temperature increases, resulting in a decrease in phenological overlap. However, start and peak dates are expected to shift differently in both species. Peak flowering and nesting were separated by an average of 2 days in 2013, but this value varied from 1 to 9 days, depending on study site. The highest projected increase in global temperatures by the end of the century (5.8°C) would increase the average asynchrony of peak phenology to 7 days. Start dates were more variable across sites, and the onset of bee activity and flowering were separated by an average of 6 days in 2013. Start date asynchrony is projected to increase to an average of 17 days with an increase of 5.8°C.

**Conclusions.** The potential impacts of an increasing phenological mismatch between the beach pea and silver bee are difficult to assess, but the consequences to these species are likely to be modest. Overlap in flowering and bee activity is likely to remain high in spite of shifts in the timing of activity. Furthermore, both species are generalists and are able to use resources from other members of the local community. Concern about the mismatch of these species should be focused on the beginning of the season, as start date asynchrony is likely to increase the most by climate warming. Pattern of shift in these species gives us insight into how pollination mutualisms in the coastal dune systems may be affected in the future. In addition, as many solitary ground nesting bee species are difficult to study at the nest site, this study may give insight into the factors that cue phenology for other similar species.
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INTRODUCTION

In the past decade, the timing of plant flowering and pollinator flight seasons has become a topic of concern due to climate change. As abiotic factors in the environment are altered, species-specific shifts in phenology may occur (Forrest and Thomson 2011). Spring phenological events have already changed in response to increases in temperature (CaraDonna et al. 2014, IPCC 2007, Menzel et al. 2006[A]). For example, ten North American bee species are now active an average of 10.4 days earlier than 130 years ago (Bartomeus et al. 2011). While the phenology of plants and pollinators have shifted together in some cases (Bartomeus et al. 2011), such synchrony may not be true in all situations. With further changes in climate, timing mismatches between plants and their pollinators may develop (Willmer 2011, Bartomeus et al. 2011). Such mismatches are more likely when plants and their pollinators respond to different abiotic cues to time their activity (Willmer 2011). However, even if plants and pollinators cue into the same abiotic factors, there is a potential for mismatch if they respond to those factors differently (Forrest and Thomson 2011). Little is known about the cues used by most species, making it difficult to predict how climate change will affect many mutualisms. Therefore, it is important to identify phenological cues in plants and pollinators that might indicate potential asynchrony.

The cuing of periodic stages in plants and animals is complex, and can involve a combination of abiotic and biotic factors. Many stimuli are known to induce flowering,
including photoperiod, precipitation, threshold temperatures, and interactions with other species (Willmer 2011). In the temperate zone, blooming is often predicted by temperature degree-day accumulation, or the cumulative amount of time spent above a particular threshold temperature (Forrest and Thomson 2011, Menzel 2002). In addition to the many types of cuing mechanisms, each plant species may respond to cues uniquely due to interactions of genotype with the local environment (Forrest and Miller-Rushing 2010). Since each plant species may respond differently, understanding how flowering time may be altered by climate changes will require predictive modeling.

The factors that cue bee emergence are less well understood. Bees undergo a period of development, and after reaching an adult stage they often go into a diapause (resting state) for a period of time before they emerge from their brood cells. Some bees undergo development through summer and enter into an adult diapause for overwintering in their natal cells (Stephen and Torchio 1961). Temperature is important in the development rate of bees (Tauber et al. 1986, Yocum et al. 2006), but its role in regulating time of emergence and flight activity varies among species. Insects often emerge when an accumulation of a specific number of degree-days causes the adult stage diapause to end (UC IPM Online 2014). Breaking of diapause has also been linked to environmental thresholds (e.g., threshold precipitation or temperature) or regulated by an internal biological clock (Forrest and Thomson 2011). Very few field studies have been done on the cuing of solitary bee phenology, especially for ground nesting bees (Forrest and Thomson 2011). Cavity nesting solitary bees are much easier to study because they will nest in blocks of wood set up by a researcher, and these blocks can then be moved and
subjected to different environmental conditions. The nests of ground nesting bees are often difficult to find and challenging to move, which makes studying the cues of emergence phenology nearly impossible. Field studies on ground nesting solitary bees are crucial to begin to understand the variables affecting their phenology.

One way to predict the cuing of flowering and emergence is based on comparing shifts in phenology with variation in climate over time. This method is difficult, as it requires detailed long-term datasets. In the absence of long-term datasets, another approach is to take advantage of existing spatial variation in phenology. In this study I took the latter approach. I determined the spatial variation in phenological events and in the timing of potential cues of phenology. I then tested whether variation in phenology is correlated to these potential cues. In this way, I used the spatial differences in abiotic variables among plots as a proxy for variation in climate over time (Forrest and Thomson 2011).

**Study species**

*Lathyrus littoralis*, or silky beach pea, is a native legume that plays an important role in the establishment and early succession of dune vegetation in the coastal dunes of Northern California (Pickart and Sawyer 1998). It is an early colonizer of open sand, utilizing nitrogen fixation and rhizomatous growth to colonize areas that later transition into the diverse dune mat community (Sawyer et al. 2009). *L. littoralis* is also one of the first plants on the dunes to bloom in the spring and likely an important early floral resource for pollinators (Gordon 1984), but little is known about the stimulus of its flowering phenology or its pollination requirements. Beach pea is a good candidate for
this study because early flowering species have been shown to be most sensitive to
cclimate changes (Menzel et al. 2006[B], Miller-Rushing et al. 2007).

*Habropoda miserabilis* (dune silver bee) is one of the most common bee species in the
dunes of Humboldt Bay. It is a generalist bee species, and is one of the first bees active in
the spring. It is known to be the most frequent visitor of *L. littoralis* (Gordon 1984), and
likely its most important pollinator. This solitary bee species nests in open sand dunes,
and creates obvious nesting aggregations (Nyoka 2004, Figure 1). These aggregations are
stable and can persist for many years (M. Mesler, K. McFarland, personal
communications). This system offers a rare opportunity to characterize the phenology of
a ground-nesting bee at the nest site. Bee species that are active early in the spring, like
*H. miserabilis*, are thought to be good indicators of phenological reactions to climate
change (Bartomeus et al. 2011).

Local biologists have noted spatial variation in the phenology of both *L. littoralis* and
*H. miserabilis*. *L. littoralis* has been observed to bloom later in the season in the northern
areas of Humboldt Bay (A. Pickart, personal communication; Monroe 2010). Likewise,
*H. miserabilis* exhibits distinct variability in flight timing among nesting aggregations
(M. Mesler, P. Haggard, K. McFarland, J. Monroe, personal communications). Flight and
nesting activity of adjacent nesting aggregations often begins and ends at different times.
Differences in flight season timing have also been reported along a latitudinal gradient
(sometimes beginning and ending almost a month earlier in the southern end of the North
Spit [Monroe 2010]). This spatial variation in both flower and bee phenology allowed for
the development of models to determine potential abiotic cues in these species.
Characterizing the phenology of pollinators is challenging. Often, flight seasons are estimated based on observations of activity on flowers. However, this characterization is not independent of the flowering time of the plant species observed, thus making it difficult to detect any mismatch between plants and pollinators unless numerous assumptions are made (Forrest and Thomson 2011). Because *H. miserabilis* females nest in groups, and each aggregation of nests occurs in the same area on the dunes for many years, I was able to characterize phenology of this pollinator at its nesting sites.

Objectives of this study were to 1) determine the pollination requirements of *L. littoralis*, and survey the pollinators of this plant species, 2) characterize current flight season of *H. miserabilis* and flowering of *L. littoralis*, and determine the degree of overlap, 3) determine the most likely cues of the onset and peak dates of bee activity and blooming, and 4) predict how the timing of *H. miserabilis* and *L. littoralis* phenology may be affected with changes in climate.
METHODS

Study sites

This study occurred in Northwestern California, along the North Spit of Humboldt Bay (40°50’28.33” N, 124°06’11.92” W). Five sites were chosen for monitoring nesting aggregations of *H. miserabilis*: Mad River County Park (MR), Lanphere Dunes Unit of the Humboldt Bay National Wildlife Refuge (LAN), the Bureau of Land Management’s Ma’lel Dunes South (MA), adjacent to the Drag Strip in Samoa (DS), and in the Eureka Dunes Protected Area and adjacent BLM Endangered Plant Protection Area (EPA) (Figure 2, Appendix A). *L. littoralis* was also monitored at five sites, but this species does not occur across the full range of the North Spit, and was not present at the two southernmost sites chosen for bee monitoring (DS and EPA). To account for this, two additional flower sites were included at the Manila Dunes Recreation Area (MAN) and Samoa Beach (SA) (Figure 2).

Pollination requirements

To determine if pollinators are required for fruit and seed set, I covered 60 inflorescences of *L. littoralis* with veils at each site (12 inflorescences at each site on 12 different plants per site). The veils were light weight and allowed for airflow, and excluded pollinators without coming into contact or pressing against flowers (Figure 3). Each veil was secured with thread, pulled tight enough to prevent insect entrance from below, but loose enough to allow for growth. The fruit set of the covered inflorescences was compared with 60 inflorescences left open to pollination (12 inflorescences at each
site). A total of 434 flowers were covered and fruit set was compared with 498 uncovered flowers.

**Pollinator surveys**

I observed visits to *L. littoralis* to determine the relative abundances of different pollinators. Every time phenology plots were visited (see next section), I counted the number of flower visits made by each type of pollinator over a 10-minute interval. All pollinator surveys took place between 11 am and 3 pm, and the order of plots visited was randomized each day. Over the entire season, I performed 180 surveys, totaling 1,800 minutes of observation.

**Characterization of flowering and nesting phenology**

To characterize the flowering phenology of *L. littoralis*, I sampled 30 circular (3 m diameter) plots at 5 sites (Figure 2). The vegetation at each site was characterized by seral stage (SS), with SS1 including *L. littoralis* and no other plant species, SS2 including *L. littoralis* and other species but <10% Poaceae, and SS3 including *L. littoralis* and other species and >10% Poaceae. Six plots at each site were chosen randomly, with the constraint that two plots per site were selected from within each seral stage class. Plots were within 1km of bee aggregations when possible.

In lieu of counting all open flowers, the number of inflorescences with at least 50% open flowers was counted on average every 6 days (s.d. 1.5 days) (Totland et al. 2006, Santos Fava et al. 2011, Norris 1996). Inflorescences were not counted after more than 50% of flowers had senesced and were no longer attractive to pollinators. Senescence
was marked by a change in banner petal color (fading from bright pink-purple to a dull blue), and keel petal color (bright white to white-brown), (K. McFarland, personal communication).

To characterize the flight season of *H. miserabilis*, I observed nesting activity at 17 aggregations chosen randomly from a pool of 41 locations documented in previous years. I chose 3 nesting aggregations per site (4 in locations with more frequent disturbance: MR and MA), and monitored them on average every 6 days (s.d. 1.5 days), varying the order of the visits on each monitoring day. Aggregations varied from 18 to 1,372 total nests over the entire season (Appendix A), and the spatial size of the aggregations ranged from approximately 10 m² to 100 m².

For each aggregation I noted the dates when males and females first emerged as well as when they were last seen at the end of the season. On each census date, I counted the number of active nests and estimated the number of males. Counting active nests entailed temporarily marking nest entrances with popsicle sticks where female activity was seen (digging, entering and exiting nests), as well as where fresh digging tracks were observed. Females dig to build their nests by pushing sand backwards, leaving distinctive patterns in the nest entrance (Figure 4, Appendix B). These patterns disappear quickly (typically within 1-24 hours after bee activity, depending on wind conditions), and are a good indication of recent activity. I spent at least two hours at each aggregation, and if any new nests were found within the last 5 minutes of this monitoring period, the interval was extended by 30 minutes. I chose a 2-hour monitoring period based on the longest recorded foraging trips for *H. miserabilis* (Monroe 2010). Male bees move quickly at nest
aggregations and are difficult to count, so the number observed at each census was binned by categories of 5 (e.g., 0, <5, 5-10, 10-15, 15-20, 20-25, etc.).

I created phenology curves to characterize the relative timing of flowering and bee foraging activity at each site as well as across the entire study area. These curves were based on the proportions of the total number of open inflorescences and active nests observed on each sampling date. To combine site counts and create overall curves for the full North Spit, the numbers of bees/flowers counted in each site over each six-day period were summed. The phenology curves were then used to calculate the percentage overlap of the two species using a modified version of the Renkonen index of niche overlap based on proportional similarity (e.g., Gross et al. 2000, Schoener 1968, Krebs 1989):

\[
\text{Percent Overlap}_i = \left( \frac{\text{total area}_i - \text{area not overlapped by } j}{\text{total area}_i} \right)
\]

Where \(i\) represents nesting, and \(j\) represents blooming (formula was repeated with \(i\) and \(j\) switched for each overlap calculation).

Emergence of new bees did not occur evenly throughout the nesting season. Because I could not directly observe all emerging bees, I estimated emergence dates based on male activity and nesting observations. To estimate the average start date of emergence, I averaged the start date of male activity and the first day I observed nesting at each aggregation. Silver bees are estimated to have approximately a 3-week lifespan (K. McFarland, M. Mesler, personal communications), so to find the approximate end date of emergence I subtracted 3 weeks from the last active nest observed. Three weeks is also consistent with estimates of lifespans of other large bees, such as worker bumblebees (Rodd et al. 1980, Goldblat and Fell 1987, Torchio 1990). To estimate the peak date of
emergence I averaged these approximate start and end dates of emergence. Although this approach is imperfect and assumes a symmetrical emergence curve, it probably gives a good approximation of when peak emergence likely occurred. I also performed all calculations based on 1 week, 2 week, and 4 week average lifespans. Because these calculations yielded similar results and essentially identical conclusions to those based on 3-week estimates, these results are not reported.

A critical assumption of this study is that bees nesting at an aggregation had emerged from the same aggregation. Although I could not verify this assumption, philopatry has been reported for other solitary bees that nest in aggregations (Soucy 2002, Potts and Willmer 1997, Yanega 1990, Sakagami and Michener 1962), and is consistent with the long-term stability of nest aggregations in my study area (M. Mesler, personal communication).

**Potential cues for flowering and bee emergence**

Air and soil temperature as well as soil moisture were measured every 6 days (s.d. 1.5 days) starting in January and continuing throughout the flight and flowering season. Soil temperature was measured in each plot at different depths (surface, and 1cm, 10cm, 25cm, and 50cm below the surface) with a Fluke 5II Thermometer probe. These depths were chosen to span the approximate range where diapausing bees are found (K. McFarland, personal communication). Soil moisture was measured just below the surface with a Decagon 5TE Soil Moisture Sensor (Decagon Devices 2014). Ambient air temperature was measured at 25cm above the surface. All of these variables were
measured in multiple locations within aggregations and flower plots, to maximize the
capture of microsite variation. Distance from the ocean was also estimated, using data
from Google Earth™ (Google Inc. 2013).

Using data from abiotic measurements, degree-day accumulation and moisture
accumulation were calculated. Accumulated degree-days were calculated as average
temperature each day (above freezing), summed each day from January 1\textsuperscript{st}. As
temperature accumulation above baseline temperatures other than 0˚ sometimes provides
better predictions for the timing of plants and insects, I also tested different degree-day
calculations, starting accumulation from other baseline temperatures above 0°C (UC IPM
ONLINE 2014, Forrest and Thomson 2011). Similarly, moisture accumulation (or
moisture-days) was calculated as the sum of the average moisture each day. Since
temperature and moisture measurements were taken every 6 days, the days in between
measurements were given the mean value of the measurements taken on the dates before
and afterwards. Possible differences in temperature accumulation between
plots/aggregations, degree-day calculations by plot/aggregation and day of the year were
compared using ANCOVA.

Taking soil moisture and temperature measurements every 6 days seemed to be
reasonable based on other studies (e.g., Warren II et al. 2011 - measurements made
weekly). However, to evaluate the merits of this strategy, soil temperature and moisture
measurements were compared with continuously recorded data in one aggregation at
Lanphere Dunes. The continuous measurements were collected every hour using a
Decagon Em30 data logger positioned at 25cm, 50cm, and 100cm (Decagon Devices 2014). Temperature sensors were rotated to different depths in the soil halfway through the season to account for any variation in measurement accuracy. To test for differences in calculations from the two different measurement types, trends in estimated accumulated degree-days by measurement type and day of the year were compared with ANCOVA. In lieu of obtaining 654 data loggers (the number of points measured every 6 days), periodic sampling seemed to give a good estimate of the temperature regimes in the soil. Leaving equipment in all sites was not an option due to permit restrictions and public access to the study sites.

**Developing predictive models**

To predict the most likely abiotic cues for the phenology of these two species, linear regression was used to determine whether the start and peak dates of phenological events were related to degree-day accumulation, moisture accumulation, or their interaction. Temperature and moisture accumulation was summed from Jan 1st until the mean day of year for each phenological response (mean across plots/aggregations). Calculating degree-days and moisture accumulation in this way standardizes the end date of the calculation for all plots/aggregations, rather than ending on the phenology response dates from individual aggregations/plots (which would be biased by the respective timing of phenology in each case). By fixing the end date of the temperature and moisture accumulation, I created a gauge of the relative warmth/moisture in flower plots and bee aggregations within a set time window (Iler et al. 2013). These measures of temperature...
and moisture accumulation were then regressed against the timing of phenology stages. Preliminary analysis revealed that site was not significant in any models and was not included as a random effect. Therefore, ordinary least squares linear regression was used to perform these analyses. Models were compared using AICc and likelihood statistics, and with ANOVA model comparisons in R.

Degree-day accumulation above freezing at the surface was used for regression models predicting flowering, and at 25 cm soil depth for models predicting bee activity. Preliminary logistic modeling analysis of flowering against predictor variables revealed that the best temperature predictor of flower blooming phenology was degree-days at 0 cm (surface) depth, with a baseline temperature of 0°C. As bee emergence curves would have to be estimated from male activity and nesting observations, modeling emergence with logistic regression would require numerous assumptions. Because of this, measurements taken at 25 cm soil depth were used to examine cues for silver bees, as this is the estimated average nesting depth (K. McFarland, personal communication). This depth estimate is similar to the average nest depths of *H. miserabilis* in other costal areas (21 cm, Bodega bay [Cane 1991]), and other *Habropoda* species (e.g., *H. laboriosa*, 28 cm [Cane 1991]). Preliminary analyses also revealed that a baseline of 0˚ worked best for predicting *H. miserabilis* emergence.

Differences in thermal regimes among plots and sites may be explained by a combination of macrosite and microsite variables. To examine the basis of differences in temperature accumulation among plots and sites, I looked for correlations between the number of degree-days reached by the average peak nesting/blooming point (averaged
across sites), and various landscape variables (slope, aspect, distance to ocean, and latitude). Slope and aspect were transformed using appropriate arctan and cosine transformations.

**Predicting the impact of temperature changes on phenology**

To predict how bee emergence and flowering might be affected by future increases in temperature, I used degree-day accumulation estimates to predict changes in the timing of *H. miserabilis* flight and *L. littoralis* bloom with projected temperature rise over the next century. To do this, I first found the mean number of degree-days required for the start and peak dates of both bee activity and flowering (an approximation of a degree day threshold, Table 5), and the average dates that this number of degree-days was reached in each site in 2013. Next, I projected increases in mean annual temperatures for coastal Northern California for the near-term (1°C, 2016-2035), mid term (1.5°C, 2046-2065), and long term (2.5°C, 2081-2100) (IPCC 2013), and the highest surface temperature rise predicted for California by the end of the century (5.8°C, Luers et al. 2006), by adding these average increases to the daily mean temperatures from each plot and aggregation collected in 2013 (Table 7). IPCC temperature projections from Dec-Feb and June-Aug were averaged, added on to the empirically observed daily temperatures, and projected degree-day accumulations from January 1st were recalculated. I then found the dates at which newly calculated degree-day accumulations reached the average number required for start and peak dates of bee activity and blooming. The dates predicted from these projections were then compared to current average start and peak dates of bee
activity/blooming.

Projected temperature increases used to predict flowering shifts were taken directly from projected average surface temperatures, whereas the projected temperature increases used to predict bee shifts were adjusted to account for buffering at 25cm below the surface of the soil. The mean reduction in daily temperatures between surface and 25cm depth was observed to be 37.9% from January-June in 2013. To implement this temperature buffering, I reduced the projected temperature increases by 37.9% before including them in the calculations for bee aggregations (Table 7). This temperature reduction was applied to make the estimates of timing shifts for silver bees more conservative.

Once the average phenology date shifts were predicted, I used them to visualize the shift in the overall phenology curves predicted with the highest surface temperature increase. Start date and peak date shifts were both incorporated into phenology curves. This was done by adding the predicted start date shift to the first date of bees and flowers observed, adding the predicted peak date shift to the overall peak date of bee activity and flowering, and adding an average of the two predicted shifts for dates in between, and the predicted peak shift to dates after the peak. From this simple graph shift, I re-calculated overlap to determine the approximate resulting change in overlap. All data analysis in this study was performed in version 3.1.1 of R (R Core Team 2014).
RESULTS

Pollination requirements

Uncovered inflorescences produced significantly more fruits than covered inflorescences (p<0.001). Only 3 of the 434 flowers that had been covered set fruit, and these fruits contained only small, flat, presumably aborted seeds. In contrast, 56.2% of the open pollinated flowers set fruit, each of which contained at least one well-formed seed.

Pollinator surveys

_H. miserabilis_ was the most common visitor to _L. littoralis_, accounting for 58.7% of all pollinator visits across sites, and performed significantly more visits ($\chi^2 = 84.6$, p < 0.0001) than the second most common visitor _Bombus vosnesenskii_ (Apidae) which made 34.3% of total visits (Figure 5). The remaining visits were made by _Apis mellifera_ (Apidae, 4.08%), _Osmia integra_ (Megachilidae, 2.53%), and _Lasioglossum pavonotum_ (Halictidae, 0.42%). All visitors except the small halictid appeared to be pollinators.

The relative importance of _H. miserabilis_ varied with date and location. It accounted for 68.5% of visits in April and May, when it was significantly more frequent than all other visitors ($\chi^2 = 959.5$, p-value < 0.0001). However, _H. miserabilis_ accounted for only 38.5% of visits in June when _B. vosnesenskii_ became significantly more common ($\chi^2 = 23.02$, p-value < 0.0001). The months when _H. miserabilis_ made over half of observed visits (April and May) coincide with the beginning and peak of the blooming season.

When pooled across months, the ratio of visits made by _H. miserabilis_ declined from
North to South. *H. miserabilis* was the most common visitor at the two most northern sites (MR [$\chi^2 = 173.2785, p < 0.001$], and LAN [$\chi^2 = 25.8736, p < 0.001$]), approximately equal in abundance to *B. vosnesenskii* at the two middle sites (MA and MAN [No significant difference in either site]), and less common than *B. vosnesenskii* at the most southern site, although not significantly less (SA [$\chi^2 = 3.4286, p = 0.064$]).

**Characterization of Flowering and Nesting Phenology**

Nesting and flowering occurred between March and July, and overlapped strongly. Blooming period overlapped 86.78% with bee nesting, and bee nesting overlapped 79.84% with blooming overall (Figure 6). The degree of overlap varied depending on the site, with the highest overlap at LAN (86.8% flower overlap with bees and 91.1% bee overlap with flowers) and lowest at MA (60.1% flower overlap with bees and 69.7% bee overlap with flowers) (Figure 7).

The start of bee activity and flowering varied from late March to early May, and activity stopped between mid June to early July. Within sites, bee aggregations varied in the timing of the start of activity between 15 and 49 days. The start dates of flowering plots within each site varied between 8 and 26 days at each site.

**Potential cues for flowering and bee emergence**

Measuring moisture and temperature at 6-day intervals turned out to be satisfactory. Temperature degree-day accumulation calculated from sampling every six days was slightly higher than with measurements every hour (Figure 8) because night temperatures lowered daily average temperatures in the continually measured sites. However, the
difference was not significant ($p = 0.507$), and the degree-day accumulation curves matched closely the trends observed from continuous measurements (Figure 8). This study could not have been accomplished in areas where soil temperatures vary greatly from day to day. The isomesic nature of the dune soils in Humboldt County allowed relatively infrequent measurements to produce very similar degree-day calculations to those created from hourly measurements. Likewise soil moisture accumulation curves calculated from 6-day vs. hourly measurements were not significantly different ($p = 0.21$).

Bee aggregations and flower plots both exhibited significant differences in temperature accumulation among aggregations/plots. The results of an ANCOVA predicting degree-day accumulation by plot and date revealed significant spatial variation in temperature accumulation ($p_{\text{date x plot/aggregation}} < 0.0001$ for both bee aggregations and flower plots).

**Predictive models**

Both bee activity and flowering were associated with climate variables. Model comparison revealed that degree-day accumulation was the best predictor of the onset and peak bee activity (Tables 1 and 2). Peak flowering was also best predicted by degree-day accumulation, but flowering onset was predicted by both degree-day accumulation and moisture (Tables 1 and 3; $p_{\text{DD:MD}} = 0.04$). The difference in AICc values was less than two for some of the flowering models, further suggesting that both degree-day accumulation and moisture may be playing a role in the cuing of *L. littoralis* (Tables 1
and 2). The inverse relationships between degree-day accumulation and the timing of bee activity and flowering suggest that the phenology of both species may advance in warmer years (Figures 9 and 10), although interactions with moisture (and potentially other cues) may moderate the advance of flowering phenology.

Latitude, distance from the ocean, and slope all influenced the differences in degree-day accumulation among plots and aggregations (Figures 11 and 12). Latitude and slope were positively correlated with the degree-day accumulation in both bee aggregations and flower plots. Distance from the ocean was positively correlated with temperature for bee aggregations, but negatively correlated in flower plots. Aspect was not correlated to differences in temperature accumulation in either bee aggregations or flower plots.

**Predicting the impact of temperature changes on phenology**

Projected warming calculations predicted that *H. miserabilis* would emerge 7.0, 9.5, 12.2, and 23.6 days earlier on average, for near-term (2016-2035), midterm (2046-2065), long-term (2081-2100) and high-end of century (2100). Average blooming start of *L. littoralis* is predicted to advance 1.9, 4.4, 7.1, and 13.2 days (Table 6). Standard errors based on degree-day threshold estimates were larger for bee predictions than for flower predictions (Table 5-7). Peak phenology dates are predicted to shift faster than onset dates for *L. littoralis*, but slower than onset dates for *H. miserabilis* (Table 6 and 7). *H. miserabilis* is predicted to reach peak activity on average 4.5, 6.4, 9.5, and 21.9 earlier for the four temperature increase projections. Peak flowering of *L. littoralis* is predicted to advance 2.0, 5.1, 8.5, and 16.9 days. Notably, in all shift predictions, blooming is
projected to shift less per unit temperature increase than bee activity. This trend holds even though temperature additions for the bee shift predictions were reduced to account for soil buffering. When temperatures were not reduced to incorporate buffering, the magnitude of the predicted mismatch was much larger. Additionally, these estimates do not include potential moderating effects of the negative interaction of moisture with temperature accumulation in cuing *L. littoralis* onset, which might further reduce the amount of shift predicted for flowering time in this species.

With the highest projected temperature increase by the end of the century (an expected average increase of 5.8°C), my models predicted *H. miserabilis* to advance its onset by an average of by 23.6 days and its peak by 21.9 days. *L. littoralis* is predicted to advance by only 13.2 and 16.9 days (onset and peak respectively). These phenology shifts would result in an increase in average asynchrony of peak phenology from its current value of 2.0 to 7.0 days. Using these predictions to shift the phenology curves of these species resulted in a reduction in area overlap of silver bees of approximately 10% (10 % less bee activity area overlapping with flowering), and a reduction for *L. littoralis* of 6% (6% less flowering area overlapping with bee activity), (Figure 13).
DISCUSSION

There is a growing concern that increases in global temperatures may result in phenological mismatches between plants and their pollinators. The number of publications covering potential mismatch and its consequences has been increasing. Some studies have shown that flowers and their pollinators seem to be keeping pace with one another (Iler et al. 2013, Bartomeus et al. 2011), while others suggest increasing asynchrony as warming continues (McKinney et al. 2012). This study suggests that the latter may occur in this system, and presents a pair of species that both respond to degree-day accumulation, but vary in their dependence on this cue.

My results show that the timing of *H. miserabilis* activity is related to degree-day accumulation. Although very few studies have been done on the cuing of the phenologies of bees, and even fewer on the cuing of solitary ground nesting bees (but see Danforth 1999), a model based on temperature accumulation fits well with known cues of insect emergence (UC IPM Online 2014), and the cuing mechanisms of other types of solitary bees (Forrest and Thomson 2011, Bosch et al. 2000, Yocum et al. 2006).

Degree-day accumulation was also the best predictor of flowering peak for *L. littoralis*, but soil moisture seems to be playing a role in flowering onset (significant temperature x moisture interaction, p = 0.044, Table 3). Additionally, the best-fit model explained only a small portion of the variation in flowering start dates (Adj. $R^2$ value = 0.084, Table 1), suggesting that *L. littoralis* may also be responding to untested cuing factors, such as day length. Plant reproductive cycles in temperate zones are often cued...
by a combination of day length and temperature (Menzel 2002). If day length is an important flowering cue for *L. littoralis*, projected increase in asynchrony with *H. miserabilis* may be even greater. Day length would moderate the dependence of *L. littoralis* on temperature as a cue for blooming by constraining flowering to a set of similar dates each year. Moderation of *L. littoralis* shift would potentially lead to an even greater mismatch between *L. littoralis* and *H. miserabilis*, as bees in diapause underground are unlikely to detect day length cues. As this was a one year study, it is impossible to test for the effects of day length on the cuing of *L. littoralis*. Further study and continued monitoring should be done to determine the relative importance of temperature and day length on the timing of flowering.

Although this study showed that the overlap of the nesting of *H. miserabilis* and flowering of *L. littoralis* is currently high, the difference in how these species respond to temperature, and the interaction of moisture cues of *L. littoralis* onset, have the potential to shift them apart with climate warming. My models predict these species to advance their phenologies with temperature increases, and an increase in temperature is predicted to have a greater impact on the timing of *H. miserabilis* (Tables 6 and 7). Projected reduction in overlap in the near future based solely on temperature accumulation is relatively small. However, warming is only one component of climate change, and changes in other environmental factors and frequency of extreme events (especially high winds) could either compound or mitigate this effect (A. Pickart, personal communication). The moderate asynchrony predicted may be exacerbated by interactions of moisture and day length cues, as both may moderate the magnitude of shift for *L.*
littoralis with climate warming. Further study is needed to assess the impacts on the
demography of these species, and whether reduced overlap would impact the population
dynamics of these species.

Start dates and peak dates are predicted to shift differently. Although estimates have
large enough 95% confidence intervals that this trend may not hold true, the prediction of
independent shifts of onset and peak dates is consistent with what has been observed in
other systems (CaraDonna et al. 2014). However, shift predictions from this study
forecast less shift in flowering start, and more in flowering peak dates, opposite of the
trend seen from long term monitoring of flowering in the rocky mountains. Shift patterns
for L. littoralis are also the opposite of those predicted for H. miserabilis, which is
projected to advance phenology more in the start than the peak phenology. These
opposing shifts may moderate asynchrony effects in the peak season, but puts special
concern for decoupling in the beginning of the season when bees may emerge earlier than
their food sources. However, since these are only rough estimates of shift, based on
simple uniform temperature increases, it too early to tell if these independent patterns in
shifting phenology will hold up.

The daily temperature experienced by silver bees prior to emergence is lower on
average than beach pea, as the bees are in diapause underground. Paradoxically, while
this may moderate temperature increases experienced by H. miserabilis as the climate
warms, it also seems to induce a more sensitive response to temperature increases.
Natural soil buffering of surface temperatures causes a reduction in the number of
degree-days accumulated each day during winter and spring. Therefore any increases in
temperatures experienced at nesting depth will result in a higher percentage increase in the overall degree-day accumulation than an equivalent increase in surface temperatures. This has implications not only for *H. miserabilis*, but also other ground nesting bee species. Previous studies on cavity nesting bee species have shown some evidence that flowering may precede bee emergence with climate warming (e.g., Forrest and Thompson 2011). This study predicts the opposite. This distinction is likely due to the different nesting biologies of the bees studied, and thus differential thermal regimes experienced during diapause. Solitary cavity nesting bees tend to overwinter in substrate above ground (e.g., dead wood and pithy stems), and degree-day accumulation in the spring is not buffered by the soil. Because cavity nesting bees are cuing into temperatures near or above the surface, degree-day accumulation would be likely to closely match or be greater than flowering degree-day accumulation (depending on the nesting substrate, height above the ground and presence of snowpack, etc.). This simple difference in where and how bees are experiencing temperatures will result in different responses to increased temperatures. Therefore, while climate warming might cause cavity nesting bees to emerge after first flowering, ground nesting bees may tend to emerge earlier in the season with climate warming. While this would rely heavily on the specific conditions of each system, the findings of this study suggest that ground nesting bees may have a higher risk of being negatively affected by phenology shifts in the beginning of the season, as their biology may make them more likely to shift faster than their host plants.

This study provides evidence that *L. littoralis* and *H. miserabilis* are strong mutualists. Results of the pollinator exclusion experiment showed that animal pollination is essential
for the fruit set of *L. littoralis*, and visitor observations confirm that *H. miserabilis* is its most frequent pollinator (Figure 5). I did not quantify the degree of dependence of silver bees on *L. littoralis*, but since *L. littoralis* is one of the first floral resources to bloom on the dunes in spring, and *H. miserabilis* is observed to use large proportions of pollen from this species, this bee is likely dependent on beach pea. In addition, the species of pollen used for nesting provisions can affect larval growth and survival even in generalist bees, and *L. littoralis* pollen may be important for *H. miserabilis* even though adult females are willing to collect pollen from a variety of plant species (Williams 2003). This mutual dependency may raise concerns about potential mismatch, but further studies will be needed to assess the potential effects that a reduction in overlap may have on the populations of these species (Enquist et al. 2014).

The effect of pollination mismatch on population demography has largely been understudied. Only a few cases have documented an impact on population vital rates, and these were mainly through a reduction in seed production (Forrest 2014). Shifts in this system would probably not have much of an effect on *L. littoralis* seed set, as reduction in overlap with *H. miserabilis* would largely be at the end of the season when bumblebees are more common. Bumblebees will likely make up for some of the reduction in pollination services due to asynchrony. Even if reduction in seed set did occur, it is not likely to be detrimental, as *L. littoralis* is a perennial plant species and is able to reproduce clonally (COSEWIC 2013). In contrast, *H. miserabilis* may be more affected by this projected shift, as both the start and peak phenology is projected to shift faster than *L. littoralis*, and bees depend on floral resources for successful reproduction.
Additionally, *H. miserabilis* may advance even more than predicted if my calculations of increase in soil temperature at 25cm are too conservative, causing a faster increase in asynchrony than expected. If floral resources are not available early in the season, any early bees would not be able to survive. While this is a concern, it would lead to a strong selection for later season bees, and not necessarily result in a long-term decline in the population (Altermatt 2010). Close monitoring at the nest site will be necessary to determine if bees are emerging before flowers in the spring, and whether any declines occur due to shift.

Developing efficient management strategies for potential pollination mismatches requires a change in perspective, since it is impossible and unrealistic to manage abiotic factors like temperature accumulation (Lawler 2009). One approach is to manage for facilitating the persistence of pollination services. Often the goal of management and conservation practices is to maintain the biodiversity and function of an ecosystem. If abiotic factors are shown to be shifting the phenology of species, it may not be crucial that original pairings of plants and pollinators are maintained, as long as the species are generalists and can move to other partners. It will be important to ensure that there continue to be sufficient floral resources throughout the flight season of pollinators, and vice versa (Aldridge et al. 2011). There is evidence that silver bees will readily use other food resources if available, and are known to visit willows (*Salix hookeriana*) and lupine species (*Lupinus arboreus, Lupinus littoralis*) flowers for food and nest provisioning. Focused management in the dunes may be applicable if phenology shifts are caused by increases in temperature. Ensuring that plants are established on a variety of slopes and
aspects may help mitigate some problems due to shifting phenology, as temperature accumulation and moisture may differ depending on these factors. It will also be important to continue the ongoing restoration practices on the dunes, as removing invasive species allows for the persistence and diversity of native flowering plants and H. miserabilis nesting locations.

Spatial variation in temperature accumulation regimes in the dunes may be a result of a combination of physical variables, both at large scales (latitude and distance from the ocean), and microsite scales (aspect and slope). Latitude, slope, and distance from the ocean all likely play roles in regulating temperatures for bee aggregations and flower plots, as all three variables were significantly correlated to degree-day accumulation in these areas. However, these tests did not examine all possible causes of variation or potential synergistic effects of these causes in degree-day accumulation. Further study will be necessary to identify all factors responsible for differences in temperature accumulation.

Although it is possible to detect likely phenology cues by careful observations and modeling, it is not always possible to detect the full complexity of these cues (Forrest and Thomson 2011). It will be important to monitor the phenology of these species over time, as long-term phenology monitoring will allow for feedback and refinement of our understanding of cuing mechanisms. Citizen scientists are currently monitoring Lathyrus littoralis phenology through the National Phenology Project and the California Phenology Project. Protocol for monitoring H. miserabilis was developed as an offshoot of this study, and is now included on the National Phenology Project (NPN) website for
monitoring (Appendix C). The *Nature’s Notebook* program has been successful in tracking the phenology of many plant and animal species through “phenophase” observations. Prior to the addition of *H. miserabilis*, the NPN phenology database included very few bee species, and protocol for ground nesting bees was entirely absent. Tracking the phenology of *H. miserabilis* based on the methods I developed will increase knowledge about how solitary ground nesting bees may respond to changes in climate.

This is the first study to characterize the phenology of solitary ground nesting bees from the nest site, and the first attempt to predict the cuing mechanism of this type of bee from abiotic field data. Phenology overlap data generated through this study can be used as a baseline to refer to when monitoring changes over time, and to assess the future synchrony of this pollination mutualism. In addition, the cuing mechanisms in these two species may be similar to other dune plant and insect species, and provide greater understanding of solitary ground nesting bee phenology and cuing in general. Insights from this study can be used to inform the management and conservation of these two species, as well as influence the management considerations of similar species.
LITERATURE CITED


Thesis work, Humboldt State University.


Botany, 94: 1470-1478.


PERSONAL COMMUNICATIONS


   Department of Biological Sciences. La Jolla, California 92093.

McFarland, Kim. 2013. Personal Communication. Master’s Candidate, Department of
   Biological Sciences, Humboldt State University, Arcata, California 95521.

Mesler, Michael R. 2012. Personal Communication. Department of Biological Sciences,
   Humboldt State University, Arcata, California 95521.

Monroe, Jane. 2012. Personal Communication. Department of Biological Sciences,
   Humboldt State University, Arcata, California 95521.

   Arcata CA, 95521.
Table 1. Linear regression models of bee activity and flowering onset in response to climate variables: degrees-days (DD, from January 1st until the mean of each phenological response), moisture accumulation (MD, soil moisture accumulation from January 1st until the mean of each phenological response), and their interaction (DD:MD). The model comparison column indicates which models performed best based on ANOVA model comparison analyses in R.

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<th>Adj R²</th>
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Table 2. Linear regression models of peak bee activity and flowering in response to climate variables: degrees-days (DD, from January 1st until the mean of each phenological response), moisture accumulation (MD, soil moisture accumulation from January 1st until the mean of each phenological response), and their interaction (DD:MD). Model comparison column indicates which models performed best based on anova model comparison analyses.

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<tr>
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</tr>
<tr>
<td></td>
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<tr>
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<tr>
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</tr>
<tr>
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</tr>
<tr>
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<tr>
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<tr>
<td></td>
<td>DD+ MD</td>
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<tr>
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<td>0.149</td>
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Table 3. Effect sizes for individual climate predictors of flowering and bee activity onset. Effects of degree-day accumulation (DD, from January 1st until the mean of each phenological response), moisture accumulation (MD, soil moisture accumulation from January 1st until the mean of each phenological response), and their interactions (DD:MD) on starting activity.

<table>
<thead>
<tr>
<th>Response</th>
<th>Model</th>
<th>Coefficient</th>
<th>Estimate ± SE</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emergence Start</td>
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<td>DD</td>
<td>-0.08 ± 0.03</td>
<td>-3.119</td>
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<tr>
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<td>MD</td>
<td>DD</td>
<td>-5.747 ± 3.4</td>
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</tr>
<tr>
<td></td>
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<td>DD</td>
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<tr>
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<tr>
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<td>DD+MD+DD:MD</td>
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<td>-0.387</td>
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<td></td>
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<td>DD:MD</td>
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</tr>
<tr>
<td>Nesting Start</td>
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</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>DD+ MD</td>
<td>DD</td>
<td>-0.09 ± 0.03</td>
<td>-3.054</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MD</td>
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<tr>
<td></td>
<td>DD+MD+DD:MD</td>
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<td>-0.33 ± 0.56</td>
<td>-0.585</td>
<td>0.568</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MD</td>
<td>-27.3 ± 5.6</td>
<td>-0.482</td>
<td>0.638</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DD:MD</td>
<td>0.02 ± 0.046</td>
<td>0.426</td>
<td>0.609</td>
</tr>
<tr>
<td>Males Start</td>
<td>DD</td>
<td>DD</td>
<td>-0.07 ± 0.02</td>
<td>-4.337</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>MD</td>
<td>MD</td>
<td>-2.69 ± 3.2</td>
<td>-0.834</td>
<td>0.418</td>
</tr>
<tr>
<td></td>
<td>DD+ MD</td>
<td>DD</td>
<td>-0.06 ± 0.02</td>
<td>-4.082</td>
<td>0.001</td>
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<tr>
<td></td>
<td></td>
<td>MD</td>
<td>-1.11 ± 2.3</td>
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<td>DD+MD+DD:MD</td>
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<td>0.12 ± 0.3</td>
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<td></td>
<td>MD</td>
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<td>0.598</td>
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<tr>
<td></td>
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<td>DD:MD</td>
<td>-0.02 ± 0.03</td>
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<td>0.575</td>
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<tr>
<td>Flowering Start</td>
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</tr>
<tr>
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<td>MD</td>
<td>MD</td>
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<tr>
<td></td>
<td></td>
<td>MD</td>
<td>-2.2 ± 2.7</td>
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<tr>
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<td>DD+MD+DD:MD</td>
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<td>0.65 ± 0.31</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>MD</td>
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<tr>
<td></td>
<td></td>
<td>DD:MD</td>
<td>-0.054 ± 0.03</td>
<td>-2.128</td>
<td>0.044</td>
</tr>
</tbody>
</table>
Table 4. Effect sizes for individual climate predictors of peak flowering and bee activity. Effects of degree-day accumulation (DD, from January 1st until the mean of each phenological response), moisture accumulation (MD, soil moisture accumulation from January 1st until the mean of each phenological response), and their interactions (DD:MD) on peak activity.

<table>
<thead>
<tr>
<th>Response</th>
<th>Model</th>
<th>Coefficient</th>
<th>Estimate ± SE</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emergence Peak</td>
<td>DD</td>
<td>DD</td>
<td>-0.05 ± 0.01</td>
<td>-4.359</td>
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<tr>
<td></td>
<td>DD</td>
<td>MD</td>
<td>-3.2 ± 1.5</td>
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<tr>
<td></td>
<td>DD</td>
<td>DD+MD</td>
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<tr>
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<td>DD</td>
<td>MD</td>
<td>0.20 ± 1.5</td>
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<td></td>
<td>DD</td>
<td>DD+MD:MD</td>
<td>-0.104 ± 0.08</td>
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<tr>
<td></td>
<td>MD</td>
<td>DD</td>
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<tr>
<td></td>
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<td>MD</td>
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<tr>
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<tr>
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<td>DD:MD</td>
<td>-0.0092 ± 0.02</td>
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Table 5. Estimated degree-day accumulation thresholds for phenology dates. Estimated thresholds are the average number of accumulated degree-days needed in 2013 to reach phenology dates.

<table>
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<tr>
<th>Type</th>
<th>Est. DD Threshold</th>
<th>Std. Error</th>
<th>95% conf.</th>
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<tbody>
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<td>Male onset</td>
<td>1267.26</td>
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<tr>
<td>Nesting onset</td>
<td>1313.72</td>
<td>56.63</td>
<td>±110.98</td>
</tr>
<tr>
<td>Emergence</td>
<td>1310.82</td>
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<td>±86.29</td>
</tr>
<tr>
<td>Flowering start</td>
<td>2429.03</td>
<td>50.52</td>
<td>±99.02</td>
</tr>
<tr>
<td>Male peak</td>
<td>1579.61</td>
<td>44.376</td>
<td>±113.60</td>
</tr>
<tr>
<td>Nesting peak</td>
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<td>±134.79</td>
</tr>
<tr>
<td>E2 peak</td>
<td>1627.72</td>
<td>65.048</td>
<td>±127.49</td>
</tr>
<tr>
<td>E3 peak</td>
<td>1652.69</td>
<td>63.43</td>
<td>±124.32</td>
</tr>
<tr>
<td>Flowering peak</td>
<td>3013.84</td>
<td>39.85</td>
<td>±78.12</td>
</tr>
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</table>
Table 6. Predicted effects of temperature increase on the average start dates of *H. miserabilis* activity and *L. littoralis* bloom. Projected temperature increases were reduced by 37.9% for bee predictions to account for buffering of temperature at 25 cm below the surface. Negative day values indicate shifts earlier in the year. Confidence intervals illustrate shift uncertainty based on the 95% confidence interval on the estimated degree-day threshold.

<table>
<thead>
<tr>
<th>Projection</th>
<th>Species</th>
<th>Increase</th>
<th>Est. Shift</th>
<th>95% Conf.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Near-Term (2016-2035)</td>
<td><em>L. littoralis</em></td>
<td>1°C</td>
<td>-1.9 days</td>
<td>± 2.4 days</td>
</tr>
<tr>
<td></td>
<td><em>H. miserabilis</em></td>
<td>0.62°C</td>
<td>-7.0 days</td>
<td>± 5.3 days</td>
</tr>
<tr>
<td>Mid-Term (2046-2065)</td>
<td><em>L. littoralis</em></td>
<td>1.5°C</td>
<td>-4.4 days</td>
<td>± 3.2 days</td>
</tr>
<tr>
<td></td>
<td><em>H. miserabilis</em></td>
<td>0.93°C</td>
<td>-9.5 days</td>
<td>± 5.4 days</td>
</tr>
<tr>
<td>Long-term (2081-2100)</td>
<td><em>L. littoralis</em></td>
<td>2.5°C</td>
<td>-7.1 days</td>
<td>± 2.3 days</td>
</tr>
<tr>
<td></td>
<td><em>H. miserabilis</em></td>
<td>1.55°C</td>
<td>-12.2 days</td>
<td>± 5.9 days</td>
</tr>
<tr>
<td>Highest Estimate (2100)</td>
<td><em>L. littoralis</em></td>
<td>5.8°C</td>
<td>-13.2 days</td>
<td>± 2.3 days</td>
</tr>
<tr>
<td></td>
<td><em>H. miserabilis</em></td>
<td>3.6°C</td>
<td>-23.6 days</td>
<td>± 5.6 days</td>
</tr>
</tbody>
</table>
Table 7. Predicted effects of temperature increase on the average peak dates of *H. miserabilis* activity and *L. littoralis* bloom. Projected temperature increases were reduced by 37.9% for bee predictions to account for buffering of temperature at 25 cm below the surface. Negative day values indicate shifts earlier in the year. Confidence intervals illustrate shift uncertainty based on the 95% confidence interval on the estimated degree-day threshold.

<table>
<thead>
<tr>
<th>Projection</th>
<th>Species</th>
<th>Increase</th>
<th>Est. Shift</th>
<th>95% conf.</th>
</tr>
</thead>
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<tr>
<td>Near-Term:</td>
<td><em>L. littoralis</em></td>
<td>1°C</td>
<td>-2.0 days</td>
<td>± 2.3 days</td>
</tr>
<tr>
<td>(2016-2035)</td>
<td><em>H. miserabilis</em></td>
<td>0.62°C</td>
<td>-4.5 days</td>
<td>± 5.6 days</td>
</tr>
<tr>
<td>Mid-Term:</td>
<td><em>L. littoralis</em></td>
<td>1.5°C</td>
<td>-5.1 days</td>
<td>± 1.9 days</td>
</tr>
<tr>
<td>(2046-2065)</td>
<td><em>H. miserabilis</em></td>
<td>0.93°C</td>
<td>-6.4 days</td>
<td>± 6.0 days</td>
</tr>
<tr>
<td>Long-Term:</td>
<td><em>L. littoralis</em></td>
<td>2.5°C</td>
<td>-8.5 days</td>
<td>± 1.9 days</td>
</tr>
<tr>
<td>(2081-2100)</td>
<td><em>H. miserabilis</em></td>
<td>1.55°C</td>
<td>-9.5 days</td>
<td>± 6.1 days</td>
</tr>
<tr>
<td>Highest Estimate:</td>
<td><em>L. littoralis</em></td>
<td>5.8°C</td>
<td>-16.9 days</td>
<td>± 1.6 days</td>
</tr>
<tr>
<td>(2100)</td>
<td><em>H. miserabilis</em></td>
<td>3.6°C</td>
<td>-21.9 days</td>
<td>± 6.0 days</td>
</tr>
</tbody>
</table>
Figure 1. Nesting aggregation of *H. miserabilis* at Ma’lel Dunes South, May 2013. Each yellow popsicle stick marks an active nest.
Figure 2. Site locations along the North Spit of Humboldt Bay. Sites with aggregations of *H. miserabilis* include Mad River Beach (MR, 4 aggregations), the Lanphere Dunes Unit of the Humboldt Bay National Wildlife Refuge (LAN, 3 aggregations), Ma’lel Dunes South (MA, 4 aggregations), The Samoa Drag Strip Area (DS, 3 aggregations), and the Eureka Protected Area (EPA, 3 aggregations). Sites with plots of *L. littoralis* include MR, LAN, MA, Manila Dunes (MAN) and Samoa Beach (SA). All sites with *L. littoralis* included 6 plots.
Figure 3. Pollinator exclosure cage. A branch of *L. littoralis* covered with veil to test if pollinators are necessary for fruit and seed set.
Figure 4. Active nest entrance of *H. miserabilis*. Note the pattern in front of entrance, consisting of parallel bands of sand pushed to the side during digging. Zigzag marks across the path are also characteristic (not shown).
Figure 5. Relative abundance of bee visitors to *L. littoralis* flowers. The number of flower visits by each species was recorded, and pooled across sites. 711 total visits were observed.
Figure 6. Phenological overlap of *H. miserabilis* nesting (blue solid line) and *L. littoralis* blooming (red dashed line) pooled across sites. Bee nesting overlapped 79.84% with blooming, while flowering overlapped 86.78% with bee nesting. Julian Date 120 = April 30th, 150 = May 30th, and 180 = June 29th.
Figure 7. Variation in phenological overlap across sites (red = flowering; blue = nesting). (A) Mad River Beach (MR), (B) Lanphere Dunes (LAN), (C) Ma’lel Dunes (MA), and (D) Southern sites (DS, EPA [bees]; SA, MAN [flowers]). Bees overlapped (A) 71.82%, (B) 86.8%, (C) 60.1%, and (D) 83.1%, with flowers, and flowers overlapped (A) 77.46%, (B) 91.10%, (C) 69.7%, and (D) 81.5%, with bees in these sites, respectively.
Figure 8. Comparison of degree-day values based on continuous recording (Δ) vs. six day intervals (+) at Lanphere Dunes. Temperatures recorded at 25cm depth. Slopes of the two lines were not significantly different (ANCOVA, p = 0.507). Temperature accumulation in both measurements increased faster near the end of the season, and were made linear through log transformation of the data.
Figure 9. Linear relationships of bee activity and flowering onset to accumulated degree-days. Solid lines indicate significant linear relationships.
Figure 10. Linear relationships of bee activity and flowering peak dates to accumulated degree-days. Solid lines indicate significant linear relationships.
Figure 11. Effect of distance from the ocean, slope and latitude on the degree-day accumulation in silver bee aggregations. Temperature accumulation was significantly correlated with (A) ocean distance ($p = 0.002$, $r = 0.70$) and (B) slope ($p = 0.011$, $r = -0.60$) and (C) latitude ($p = 0.0004$, $r = -0.76$). Aspect was not significantly correlated with degree-day accumulation.
Figure 12. Effect of distance from the ocean, slope and latitude on the degree-day accumulation in flower plots. Temperature accumulation was significantly correlated with (A) ocean distance (p = 0.026, r = -0.42) and (B) slope (p = 0.011, r = -0.14) and (C) latitude (p = 0.0004, r = -0.21). Aspect was not significantly correlated with degree-day accumulation.
Figure 13. Predicted shifts with temperature increases. Overall phenology with (A) current average temperatures and (B) the projected high temperature increase of 5.8°C by the end of the century. Flowering (red dashed line) and bee nesting (solid blue line) phenology curves are overlaid. The amount of bee overlap with flowers decreased from 79.8% to 68.7%, and the amount of flower overlap with bees decreased from 86.8% to 80.3% with the projected shift in graph (B). This is an overall decrease in overlap of approximately 10% for *H. miserabilis*, and approximately 6% for *L. littoralis*. 
APPENDIX A

AGGREGATION INFORMATION

Table 1. Location and size of bee aggregations. Location information is in WIGS 84 UTM. Start refers to the first date bees were seen active at the aggregation, and end is the last bee activity seen at the aggregation (date of last active nest) in 2013. Size refers to the total number of nests counted over the entire season in 2013. Aggregation size was not significantly related to bee phenology dates.

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APPENDIX B

NATURAL HISTORY NOTES

Emergence

Bees dig straight up when emerging out of the sand. Often other already emerged male bees will help other bees emerge by removing sand from the surface. These helper bees usually dig frantically into the sand, presumably to be the first to mate with the emerging virgin females. As soon as the sex of the bee emerging is detected (when emerging bee is just about to break free of the sand) the “helper” bees either leave promptly (if they discover it is male), or continue to dig frantically and attempt to mate (if they discover it is female). How these helper bees know the precise location of these emerging bees, or when exactly they determine the sex of these bees (e.g., visual or pheromone cues) is unknown. Newly emerging male bees often have the luxury of staying in the newly open hole and looking out for some minutes before deciding to fly. Once emerging bees exit, they leave behind open holes in the ground. These holes are distinct from nest entrance holes as they are symmetrically round from above, and go straight down into the sand (not at an angle), (K. McFarland, personal communication).

Choosing a nest location

A female bee’s decision about where to start her nest takes time. Females will look around for hours, maybe even days, before a nest location is selected. If other nests are present at the aggregation, females will often fly around to examine, and sometimes enter the nests of other females before deciding on a nest location themselves. Female bees
examining other nests rarely actually walk into the nest, preferring to remain near the entrance and looking in. They tend to examine other nests only when the females that “own” the nest are not present.

**Nest digging**

Nests are dug little by little as the female walks quickly forward into the nest, and then pushes sand out as she works her way backwards out of the nest. Often she will make a short buzzing noise, almost like a chirping sound, when she changes directions from digging backwards to walking forwards into the nest again. Every so often, while digging, she will fly out and look back on the nest, as if to take a step back to admire her work and remember landmarks around the entrance hole. This occurs more frequently when the female is just starting to dig. Once the nest is established and she begins to collect pollen, more digging will occur if the nest entrance degrades due to wind action, collapses or needs to be repaired.

Typically females dig their nests into slopes, digging sand backwards out of the hole down the slope. If the slope is steep, gravity takes over and pulls sand downward and away from the nest entrance. However, usually females have to push the sand away from the nest entrance themselves, and typically push the sand away from the entrance hole 10-20cm, often arching away from the nest to the left or right.

**Relocating nests**

When returning to their nests, females are very cautious about finding the correct nests. They will double check landmarks, and stop to look into the entrance hole before
entering. This is done to avoid entering the incorrect nest. This behavior is especially noticeable when something about the landmarks has changed: new nests are present, old nests are gone, any changes in the plants, changes in deep footprints nearby etc. When nests are obscured in any way (e.g., by sand covering part of the nest entrances) females may circle the nest looking at landmarks, land to look closely at the nest entrance, and repeat this process until she is sure the nest is hers.

If the nest entrance is completely covered or collapsed, the female will search by circling and locating the approximate location of the old nest, landing to try digging, and then repeating the process. Often she will be very frustrated trying to reform the nest entrance, and will vocalize her frustration with a series of buzzing noises.

**Plants used for food and nest provisions**

*Habropoda miserabilis* can be seen visiting willows (*Salix hookeriana*) in early spring, evergreen huckleberry (*Vaccinium ovatum*), bearberry (*Arctostaphylos uva-ursi*), silky beach pea (*Lathyrus littoralis*), strawberry (*Fragaria chiloensis*), beach evening primrose (*Camissonia cheiranthifolia*), morning glory (*Calystegia soldanella*), lupine (the native *Lupinus littoralis*, and invasive *Lupinus arboreus*), yellow sand verbena (*Abronia latifolia*), sea rocket (*Cakile edentula* and *Cakile maritima*) and weedy mustards (*Brassica rapa*).

**Pollination of Lathyrus littoralis**

Although differences in pollen deposition between *H. miserabilis* and other bee species was not experimentally tested, based on the observations performed by this study,
it can be assumed that silver bees do contribute substantially to pollination of *L. littoralis*. Only one *Lasioglossum pavonotum* individual was observed on *L. littoralis* flowers very early in the season attempting to reach pollen and nectar. As it is such a small bee species it did not seem to be able to effectively manipulate the flowers to pollinate. The other three bee species observed were large enough to manipulate the flowers for effective pollination, however *B. vosnesenskii* was observed robbing nectar in some instances, biting a hole in the bottom of the corolla to access nectar. *Osmia integra*, a legume specialist, was only observed once during the flowering period, and only in a restored and minimally disturbed site (Lanphere Dunes). *Bombus mixtus* was also seen visiting flowers once in late May, but was not observed during any observation periods and therefore not included in the results.

**Invasive species**

Invasive species in the dunes impact silver bees and other bees in the dunes in a variety of ways. Invasive plants directly impact silver bees, and other ground nesting bees, by densely covering sand dunes and eliminating areas to nest. Some of the aggregations I visited in Mad River Beach and the Samoa Drag Strip are becoming densely covered with the invasive grass *Ammophila arenaria*. The bees will not nest where *A. arenaria* or invasive annual grasses are dense. In addition, when nesting near invasive grass stands female bees often become entangled in the many fine roots while digging their nests. Although the encroachment of invasive grasses onto an aggregation
will cause bees to shift or shrink the area where they are nesting, bees will begin to “recolonize” and nest in the invaded areas quickly after grasses have been removed.

*Lupinus arboreus* provides pollen resources for bees, especially at the southern end of the North Spit where *Lathyrus* is not present. Although its presence in the landscape is often detrimental to nesting aggregations, densely invading itself and allowing for the encroachment of invasive annual grasses, it seems to be an important pollen resource in unrestored areas with very few other flowering species. This should be a consideration during restoration practices at the southern end of the spit, as *H. miserabilis* would likely benefit from planting native flowering species upon the removal of *L. arboreus*.

Invasive argentine ants also impact the silver bees. I noticed this especially in the area adjacent to the Samoa Drag Strip, although a few ants were seen to lesser extent in other areas. The ants were not present in the very beginning of the nesting season. However, as soon as bee nest density increased near the peak of the season at the Samoa Drag Strip, the ants seemed to be a significant problem for the female bees. The ants would congregate around nest entrances, walking down into the nests and grabbing on to females as they tried to enter. Females were obviously bothered and deterred from entering their nests, making loud buzzing sounds and trying to avoid the ants as they entered their nests. The time it took for the females to enter their nests increased greatly when ants were present, as they would hesitate before entering and struggle as they tried to walk into their nests. It is unclear how widespread these ants are on the dunes, or how they might affect populations of *H. miserabilis* in the area, but were observed in all sites (in small numbers except in nesting aggregations at the Samoa Drag Strip). Argentine
ants are often associated with dense willow patches, and areas with a good amount of moisture (D. Holway, personal communication), and the most intense infestations were nearby willows. Spread of these ants should be monitored, and management actions should be taken to reduce spread of these ants. Management may require direct application of pesticides into the nests, (D. Holway, personal communication). It is important to prevent spread of these ants, as it is difficult to remove them once they are established. Argentine ants have been observed in association with disappearance of ground nesting bee species in other areas (D. Holway, personal communication).
APPENDIX C

INFORMATION AND METHODS FOR MONITORING THE PHENOLOGY OF

*H. MISERABILIS*

The following is information and methods for monitoring the flight season phenology of *H. miserabilis*, through the National Phenology Network (NPN). All of this information can also be found on the NPN citizen science program website, *Nature’s Notebook*, (*H. miserabilis* 2014). Data for this monitoring program is collected on data sheets and then entered by participants into the website, along with the location of the observations. Participants with smart phones can also enter data directly to the website as they record it through the *Nature’s Notebook* app (USA NPN 2014).

*What does this species look like?*

Silver bees are large, robust bees, with grey-black coloration. They have two sets of transparent wings with black veins. The front set of wings is larger than the hind set. Females carry pollen on their hind legs. The body is covered with silver-grey hair; the body color below the hairs is black.

Similar species: silver bees may be confused with bembix wasps. Bembix wasps have shiny, smooth, abdomens, with white to blue-yellow stripes, whereas silver bees have a fuzzy abdomen with grey stripes. Bembix wasps dig by rapidly flicking the sand backwards, silver bees do not flick the sand (they dig with sweeping movements of their legs). Silver bees also tend to look shorter and fatter with black eyes, while bembix wasps are long and narrow with blue-green eyes.
General phenology and life history

Silver bees overwinter underground as adults. Males emerge first in the spring, as early as February or March, and begin to look for females. Soon after the first males appear, females emerge from the ground and mate. Mating is often a big group of males fighting over the female, and wrestling on the ground. After mating, females begin to dig nests in the soil. Each female bee digs her own nest, but they tend to nest in groups, and usually many nest entrances (holes) will be seen in one area. Each nest starts as one tunnel, which branches underground into a few side tunnels. At the end of each of these tunnels she creates a space called the “brood cell,” where she stores pollen and nectar from the flowers she visits. After the brood cell is filled with a ball of pollen and nectar, the female will lay an egg, fill the tunnel with soil, and then begin a new tunnel. Fertilized eggs turn into females and unfertilized eggs, usually produced late in the season, become males. A female bee will continue to create tunnels, provision brood cells and lay eggs until she dies. By the end of the season, around early July, all adult bees die. The eggs laid during the season will hatch, eat and develop into larvae, then into pupae and finally into adult bees. These new adult bees will remain underground, in a state called “diapause,” through the winter until spring comes again.

Special considerations for observing

Nest entrances are fragile and can collapse easily. Please watch where you step while monitoring! Some areas also require special permissions, so please find out if there are any requirements before beginning to monitor.
Directions

As you report on phenophase status (Y, N or ?), refer to the definitions below to find out what you should look for, for each phenophase. For reporting animal abundance, if a specific question is included below the phenophase, choose the best answer to the question. If there is no specific question, enter the number of individual animals you observed in each phenophase. Feel free not to report on phenophases or abundances if they seem too difficult or time-consuming.

Which phenophases should I observe?

Active adults
One or more adults are seen moving about or at rest.
For abundance, enter the number of individual animals observed in this phenophase.

Flower visitation
One or more individuals are seen visiting flowers or flying from flower to flower. If possible, record the name of the plant or describe it in the comments field.
For abundance, enter the number of individual animals observed in this phenophase.

Male individuals
One or more males are seen moving about or at rest. For Habropoda miserabilis, males have bright white faces and are generally seen flying low to the ground searching for females.
For abundance, enter the number of individual animals observed in this phenophase.

Mating
A male and female are seen coupled in a mating position, usually with the male on top of the female. For Habropoda miserabilis, multiple males may swarm a female, fighting to mate with her on the ground. This can look like a “ball of bees” wrestling on the ground.
For abundance, enter the number of individual animals observed in this phenophase.
Nest building
One or more adults are seen digging into soil to construct a nest. For *Habropoda miserabilis*, adult females walk quickly forward into the nest, then inch back out, removing soil with sweeping movements of their back legs.

*For abundance, enter the number of individual animals observed in this phenophase.*

Nest provisioning
One or more adults are seen entering the nest with pollen. For *Habropoda miserabilis*, pollen is carried on the back legs, appearing as bright yellow, orange, or white coloration of their back legs.

*For abundance, enter the number of individual animals observed in this phenophase.*

Dead adults
One or more dead adults are seen, including those found on roads.

*For abundance, enter the number of individual animals observed in this phenophase.*