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Geographic Variation in Blue Orchard Bee (*Osmia lignaria*) Development and Performance as a Managed Pollinator in the Western United States

Morgan B. Scalici
Utah State University, morgan.dunn@usu.edu

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GEOGRAPHIC VARIATION IN BLUE ORCHARD BEE (*OSMIA LIGNARIA*) DEVELOPMENT AND PERFORMANCE AS A MANAGED POLLINATOR IN THE WESTERN UNITED STATES

by

Morgan B. Scalici

A thesis submitted in partial fulfillment
of the requirements for the degree

of

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Ecology

Approved:

Diane Alston, Ph.D. Major Professor	Theresa Pitts-Singer, Ph.D. Committee Member
Matt Yost, Ph.D. Committee Member	D. Richard Cutler, Ph.D. Vice Provost for Graduate Studies

UTAH STATE UNIVERSITY
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ABSTRACT

Geographic variation in blue orchard bee development and performance as a managed pollinator in the western United States

by

Morgan B. Scalici, Master of Science

Utah State University, 2023

Major Professors: Dr. Diane Alston, Dr. Theresa Pitts-Singer, Dr. Matt Yost
Department: Biology

The blue orchard bee (Hymenoptera: Megachilidae: *Osmia lignaria*) is North America’s most agriculturally important native mason bee as an effective pollinator of spring-blooming fruit crops. Differences in developmental phenology between geographically distinct populations, in ways that impact their management, have recently been identified. Populations from northern Utah, where the bee was originally domesticated, have been the most well-studied and employed as pollinators. Much about how other populations differ in their development, reproduction, and nesting behavior is largely unknown. In the studies reported here, I compare development and post-emergence performance of blue orchard bees sourced from Utah with bees from other western locations. In Chapter I, I compared in-orchard performance by California- and Utah-sourced bees in both states. Bees performed similarly in Utah orchards, but twice as many Utah-sourced females were observed in California orchards than were California-sourced females. The presence or absence of unmarked bees revealed that local bees were likely absent in California orchards but abundant in Utah orchards, and is a proposed factor for the high population return in Utah orchards. In Chapter II, I reared Utah- and Washington-sourced bees under constant and natural thermal regimens to compare effects on development and emergence. I found that development differed when these bee populations were reared at the same constant temperatures, and that both populations suffered from exposure to maximum temperatures in their respective orchard-growing regions. Female bees from both populations emerged in synchrony with local crop bloom at natural temperatures, but the emergence period was prolonged compared the quick emergence period of bees in the constant thermal regimen. In Chapter III, I flew California- and Utah-sourced bees in screened field cages to examine the impacts of pairing geographically distinct phenotypes on development, emergence, and post-emergence performance. Bees from UT were twice as likely to establish nests than bees from CA, but nesting time and reproductive output of nesting females was similar. No disruption in male-female emergence timing was found in offspring of mixed population crosses. This research highlights novel differences in geographically distinct blue orchard bee populations and supports previous research that proposed a genetic basis for regional characteristics.

(137 pages)

PUBLIC ABSTRACT

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Most flowering plants, including many cultivated food crops, will only produce well-developed fruits and seeds if pollen is transferred from one flower to another with the help of an animal pollinator. Honey bees are the most well-known and ubiquitous but are poor pollinators of some commercially important crops, or are in poor supply during crop bloom. In such cases, farmers will employ other managed pollinators such as bumble bees or solitary bees like mason and leafcutting bees. The blue orchard bee is North America's most agriculturally important native mason bee as effective pollinators of spring-blooming fruit crops. Differences in developmental biology between geographically distinct populations, in ways that impact their management, have recently been identified. Populations from northern Utah, where the bee was originally domesticated, have been the most well-studied and employed as pollinators. Much about how other populations differ in their development, reproduction, and nesting behavior is largely unknown. In the studies reported here, I compared development and post-emergence performance of blue orchard bees sourced from Utah with bees sourced from other western locations. In Chapter I, I compared in-orchard performance by California- and Utah-sourced bees in both states. Bees performed similarly in Utah orchards, but twice as many Utah-sourced females were observed in California orchards than were

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Morgan B. Scalici

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

INTRODUCTION

Pollination of flowering plants (Angiospermae) is essential for life on Earth as we know it. Defined as “the transfer of pollen from the anther of one flower to the stigma of the same or another flower”, pollination induces fruit and seed production in flowering plants (Abrol 2015). While some plant species can be pollinated by wind or water, most flowering plants rely on animal mediators to various extents for their sexual reproduction and/or fruit production (McGregor 1976, Klein et al. 2007, Ollerton et al. 2011). Thus, pollinators of wild and cultivated plants play a key role in sustaining diverse ecosystems and aiding in the production of food crops that nourish humanity across the globe.

Although numerous animal taxa visit flowers to feed on nectar and/or pollen, bees (charismatic insects belonging to the clade Anthophila [Hymenoptera: Apoidea]) are the primary group of organisms that actively gather pollen as a food source for their young (Wilson and Carril 2016). Their reproductive success (the number of offspring that survive to adulthood) is largely dependent on their ability to collect pollen (Minckley et al. 1994, Ganser et al. 2021). As pollen-collecting specialists, the life cycle and ecology of bees are intimately tied to flowering plants. Aspects of their anatomy (e.g., plumose setae), physiology (e.g., voltinism), and behavior (e.g., floral constancy) make bees supreme pollinators. As such, bees are the only organisms to have been domesticated specifically for pollination of food, fiber, or fuel crops.

An estimated one-third of the typical human diet consists of foods or building blocks to foods that benefit from bee pollination – namely, fruit, seed, and leguminous forage crops (McGregor 1976, Klein et al. 2007). Pollinator-dependent food crops (e.g., apple, tomato, and sunflower) are rich in flavor, essential nutrients, medicinal use, cultural significance, and economic value (Olcott-Reid and Reid 2007, Abrol 2015). The global cultivation and production of pollinator-dependent crops has increased over the last several decades and so has the demand for pollination services (Aizen et al. 2008, Aizen and Harder 2009, Calderone 2012).

Modern agroecosystems often lack the wild pollinators needed for profitable fruit or seed set in pollinator-dependent crops. Thus, it becomes necessary to introduce managed pollinators into crop fields for their blooming period (Kremen 2008). The western honey bee (*Apis mellifera* Linnaeus) has historically been the sole managed pollinator available to aid in the production of most pollinator-dependent crops (McGregor 1976). The typical scenario is that farmers contract beekeepers, paying a pollination service fee based on the agreed upon number and strength of colonies placed in the cultivated fields during crop bloom (Ferrier et al. 2018). Beekeepers in the U.S. transport honey bee colonies across the country to various crops where and when they are needed to satisfy the growing demand for pollination services (Bond et al. 2021).

However, honey bee populations in the United States have declined over the past several decades and pollination fees have soared (Aizen and Harder 2009, vanEngelsdorp and Meixner 2010, Ward et al. 2010, Bond et al. 2014, Kulhanek et al. 2017). Important causes of mortality are attributed to varroa mites (*Varroa destructor* Anderson & Trueman) and the viruses they vector, other pests and pathogens, pesticide exposure, loss of foraging habitat, and long-distance transportation (Naug 2009, Bromenshenk et al. 2010, Carrek et al. 2010, Straub et al. 2016, Bond et al. 2021). Honey bee populations will likely not be able to support the global increase in agricultural dependence on pollinators, even in regions of the world where honey bee colony numbers are increasing

(Aizen and Harder 2009, vanEnglesdorp and Meixner 2010). Additionally, honey bees are suboptimal pollinators of some crops including alfalfa and tomato (Cane 2002, Cooley and Vallejo-Marín 2021).

Reliance on one bee species as the sole crop pollinator is risky from both food security and environmental perspectives. Alternative (non-*Apis* spp.) pollinators are needed to sustain economically viable and environmentally sustainable crop production. In addition to providing habitat for wild bees, one strategy to assure profitable yields of pollinator-dependent crops is to employ other bee species that are also being commercially managed for pollination services (Isaacs et al. 2017). For example, several bumble bees (*Bombus* spp.) and megachilid bees (Megachilidae), such as the alfalfa leafcutting bee (*Megachile rotundata* Fabricius) and mason bees (*Osmia* spp.), have been developed for pollination of various crops (Bosch and Kemp 2002, Velthuis and vanDoorn 2006, Pitts-Singer and Cane 2011, Peterson and Artz 2014). Seasonality, foraging preference and behavior, pollinating efficacy, life cycle, and developmental biology are all important factors when determining if a species might be a suitable pollinator for a particular crop (Bosch and Kemp 2002).

Megachilid bees include the most cosmopolitan and agriculturally important solitary bees. Unlike the majority of bee species that nest underground, most megachilids nest in aboveground, pre-existing cavities – an invaluable trait for mass rearing, translocating, and utilizing artificial nesting structures in prescribed environments (Peterson and Artz 2014, Harmon-Threatt 2020). Mason bees are particularly useful for pollinating spring-blooming fruit trees. The Japanese hornfaced bee (*O. cornifrons* Radoszkowski) is an essential pollinator for apple production in Japan and demonstrates the recent development of a successful mason bee industry (Sekita 2001, Osterman et al. 2021). The utility for the European orchard bee (*O. cornuta* Latreille) and the red mason bee (*O. bicornis* Linnaeus) as a commercially manageable pollinator has been demonstrated in Europe for pollinating rosaceous fruit crops (Vicens and Bosch 2000, Gruber et al. 2011, Sedivy and Dorn 2013). In the United States, the blue orchard bee (*O. lignaria* Say) is increasingly employed as a managed pollinator of various spring-blooming fruit crops, primarily almond and cherry (Bosch and Kemp 2001).

The availability of crop pollinators for spring-blooming fruit trees is especially concerning because of their economic value, high flowering density, self-incompatible pollen, frequent inclement weather conditions, and limited availability of pollinators during the blooming period (Abrol 2015, Reilly et al. 2020). For example, almond production in California is valued at over 5 billion U.S. dollars per year and accounts for over 80% of all pollination service fees collected in the United States (USDA-NASS 2023). Almond pollination requirements are high because 90-100% of the flowers must be cross-pollinated in order to produce profitable yields (Abrol 2015). Also, the early flowering period (February-March) coincides with cool weather and a dearth in honey bee and wild bee activity (Delaplane and Mayer 2000). The cost of preparing honey bee colonies in late winter and transporting them to the Central Valley also increase the cost of pollination service fees, potentially at the detriment to colony health and beekeeper income (Bond et al. 2021).

Utilizing blue orchard bees and honey bees as co-pollinators could help relieve pressure on beekeepers to meet high demands for pollination services in crops like almond. For growers, the addition of blue orchard bees makes for a more robust and reliable pollination system with the potential to increase fruit set (Bosch et al. 2006, Brittain et al. 2013, Pitt-Singer et al. 2018). As a new and developing industry, using blue orchard bees for crop pollination has not yet scaled to levels that can replace honey bees in large-scale operations; rather, both bees should be co-deployed with a reduced honey bee stocking rate. For small acreage on a heterogeneous landscape, using blue orchard bees alone can meet the pollination requirements of most fruit tree crops (Stephen 2003).

Blue orchard bees are well suited to management for pollination services in commercial orchard crops because they readily nest in artificial materials, are naturally active in the spring, and prefer to forage on plants in the rose family (Rosaceae) such as apple, almond, cherry, pear, and plum (Bosch and Kemp 2001). Field trials have repeatedly demonstrated their pollinating efficacy and a positive return on bee populations in several fruit tree and bush crops (Torchio 1976, 1981, 1985, Bosch and Kemp 1999, 2001, Bosch et al. 2006, Boyle and Pitts-Singer 2016, 2019, Andrikopoulos and Cane 2018).

Similar to the pollination model used for the alfalfa leafcutting bee (and unlike contracting honey bee pollination services), blue orchard bees are typically purchased and managed by the grower (Peterson and Artz 2014). Growers new to keeping mason bees should work with a pollination consultant for the first few years. Thus, the initial costs of using blue orchard bees, including nesting materials, is high but yearly costs are typically low once a managed population has been established. With the production of a more reliable supply of bees and improved mass rearing equipment and techniques, costs will decrease and utilizing blue orchard bees for pollination services will become more attractive to growers.

The added task of managing blue orchard bees can be feasible for growers because it requires far less input compared to honey bees. These inputs include nesting materials, an environment with abundant floral resources and moist soil for bee reproduction during the nesting season, and a facility for storage (climate-controlled if bees are not reliably managed at ambient temperatures). Blue orchard bees are univoltine (produce one generation per year) and nesting females live for only 2-4 weeks (Bosch and Kemp 2001). Thus, free-flying adults are active for just a short time period each spring; the rest of their lives are spent within their nest and/or cocoon, and little intervention is needed (see Bosch and Kemp 2001 and Bosch et al. 2008 for a detailed description of the life cycle and management practices).

Nesting materials consist of two important components, linear nesting cavities of appropriate dimensions and a shelter to house and protect the nesting cavities from harsh environmental conditions. Nesting cavities may be constructed of various materials, most commonly used are wooden laminates (grooved boards), cardboard tubes with paper straw inserts, and hollowed stems (e.g., *Phragmites* spp.). Shelters should be made from durable and reusable materials, such as plastic storage containers that can be mounted on T-posts within orchard rows and corrugated plastic crates that can be zip-tied to tree branches. Wooden laminates are currently the most popular choice for mass-rearing because they can be sanitized and reused for several years and because the cleaning process can easily be scaled from hand- to machine-stripping of nests as operations grow.

Within nesting cavities, blue orchard bees build a series of brood cells comprised of a single egg laid on a food provision (mixture of nectar and pollen) encapsulated by soil partitions. Under favorable conditions, females can provision upwards of 30 brood cells (Bosch and Kemp 2001). However, in commercial orchards, reproductive potential is typically limited to 2-4 nests, each containing an average of five brood cells (Bosch and Kemp 2001). The slower-developing females are laid at back of the nest, so as not to be disturbed by the earlier-emerging males toward the nest entrance. The typical sex ratio for blue orchard bees is 1.5-2 males per female as found in both managed and wild-trapped populations (Bosch and Kemp 2001).

Because the free-flying adult lifespan and the duration of bloom for most fruit tree crops are similar, blue orchard bees are typically used for only one pollination event each spring. However, due to individual variation in emergence timing and the potential for staggered bee releases, bee activity can often last longer than crop bloom. Populations can be successfully translocated for pollination services elsewhere with the aid of a large visual landmark near the nesting structure(s) (Bosch and Kemp 2001). Alternatively, ensuring that supplemental forage is available before and especially after bloom can enhance reproductive output, and thus, increase the number of bees available for pollination the following year (Boyle et al. 2020).

After crop bloom and bee activity has ceased, nesting materials and the contained next generation of bees can be removed from crop fields and any necessary management steps can be taken such as storing them at particular temperatures for optimal development (Bosch et al. 2008). After immature bees develop into cocooned adults, nesting materials and bee cocoons should be sanitized to control pests and diseases. This “loose cell system” also allows bee managers to assess population growth (or deficit) and for compact winter storage; this system is largely modeked on the practices used to manage the alfalfa leafcutting bee (see Pitts-Singer and Cane 2011). Cocooned adult bees can then be stored, in a protected outdoor enclosure or temperature-controlled chamber such as a standard refrigerator, until they are needed for pollination the following spring.

Additional benefits to using blue orchard bees are that they are non-aggressive, meaning the chance of stings are low, and typically forage within 100 m of their nest which provides a safer environment for farm laborers and surrounding developments. Blue orchard bees can easily be translocated from supplier to grower by shipping cocooned adults with cold packs; the movement of blue orchard bees in this way contrasts with trucking honey bee hives long distances and requires far less input of fuel, machinery, and labor for hauling and loading. If blue orchard bees are sourced locally or regionally, bee managers may be able to leave bees under ambient conditions or reduce the time needed in climate-controlled storage (Bosch and Kemp 2000).

Also, because blue orchard bees are more effective per female than honey bees at pollinating orchard crops, fewer individuals are needed. Their increased efficacy can be attributed to their behavior, physiology, and phenology. Blue orchard bees simultaneously gather nectar and pollen from flowers, and have an irregular foraging behavior that effectively cross-pollinates the main crop variety and pollinizer varieties, if present (Bosch and Kemp 2001). The blue orchard bee transports pollen dry (not mixed with nectar) on specialized hairs on the underside of the abdomen (called a scopa) which consistently makes direct contact with the reproductive parts of the flower (Bosch and Kemp 2001). Blue orchard bees are spring-adapted, which allows them to be one of the earliest-emerging insects and to fly in cooler and cloudier temperatures than honey bees (Bosch and Kemp 2001).

The timing of life cycle events (i.e., bee phenology) varies depending on geographic location (Bosch et al. 2008). For example, spring emergence and bee activity corresponds with warming temperatures and flowering of early-blooming trees, shrubs, and herbaceous plants. Blue orchard bees may emerge as early as February in warm climates or as late as June in cold climates with high elevation (Bosch and Kemp 2001). So while their natural activity period overlaps with the bloom time of many orchard crops, established cold winter storage and warm spring incubation practices are often used to manipulate emergence timing (Bosch and Kemp 2000). Controlling emergence and subsequent nesting allows growers and managers to synchronize bee activity with crop bloom, so as to maximize both pollination (and in turn, fruit set) and bee reproduction.

The geographic distribution of the blue orchard bee is wide, occurring throughout much of the United States and southern Canada in two distinct subspecies: *O. l. propinqua* Cresson west of the 100th meridian and *O. l. lignaria* Say to the east (Rust 1974). Most of the bee stock for commercial use is trapped and/or propagated utilizing native populations throughout the western United States, as limited efforts have been made to increase managed populations in orchards by planting flowering ground cover to provide resources just for bee production. Blue orchard bees were first identified as manageable orchard pollinator by USDA-ARS scientists in Logan, Utah in the 1970’s; consequently, populations sourced from nearby montane wildlands (where bees are abundant) have been the most widely studied and employed as pollinators (Torchio 1976). In an effort to produce a commercial supply, bees trapped in Washington, Oregon, Idaho, and California are also being used for crop pollination, including transport to potentially novel climates.

Inasmuch, one constraint to a more widespread commercial use of blue orchard bees is that populations from different climates exhibit regional variation in developmental phenology (Sgolastra et al. 2012, Pitts-Singer et al. 2014). For instance, in order to cope with the extended warm temperatures in their native range, California bees have a longer prepupal (cocooned 5th stadium larva) summer aestivation compared to bees natal to Utah and Washington (Bosch et al. 2000, Pitts-Singer et al. 2014). If Utah and Washington bees produce offspring in California during February-March almond bloom, and if they remain under ambient California conditions, they become adults earlier in the summer than bees natal to California and their fat reserves are depleted before temperatures decline to initiate winter diapause in autumn and winter (Sgolastra et al. 2011, Pitts-Singer et al. 2014). Bees with depleted fat stores are unlikely to survive the winter.

Thus, bees from cooler climates, where the majority of commercial blue orchard bee populations are sourced (Utah, Washington, Oregon, Idaho) must be managed at artificial temperatures for much of the year in order to survive in a warmer climate such as in central California (Bosch et al. 2000). The difference in developmental phenology may in part be under genetic control, suggesting geographic variation in climatic adaptation (Bosch et al. 2000, Pitts-Singer et al. 2014). Due to this regional variation, the natal origin of bees should inform rearing practices.

Furthermore, there are early indicators of reduced fitness when geographically distinct populations are mixed (Glen Trostle, pers. comm.). However, the existence of local behavioral adaptations of these bees are unknown, such as the propensity to nest in provided materials and forage among target orchard flowers, the ability of females to reach reproductive potential, and the survival of offspring. While there are some documented differences between blue orchard bee populations in the western United States, a full understanding of these differences will permit the development of best management practices for crops that occur in different places and times using bees from various geographical sources. It will also allow bee producers and orchardists to make informed decisions for sustaining bee stocks that may contain advantageous characteristics for crop pollination.

Here, I examine variation in the developmental phenology and post-emergence performance of geographically distinct blue orchard bee populations in the western United States. I aim to:

1. Compare nest establishment (female retention and dispersal) of California- and Utah-sourced blue orchard bees released in cherry orchards in both states to determine if bee origin affects in-orchard performance (see Chapter I).
2. Determine variation in developmental phenology and emergence of Utah- and Washington-sourced bees when reared under constant and natural thermal regimens (see Chapter II).
3. Examine effects of mixing blue orchard bee populations (of California and Utah origin) on development, emergence, and post-emergent performance when flown in screened field cages (see Chapter III).

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CHAPTER I

GEOGRAPHIC ORIGIN AND ORCHARD GROWING REGION AFFECT BLUE ORCHARD BEE NEST ESTABLISHMENT AT ARTIFICIAL NESTING SITES

Abstract

The blue orchard bee (*Osmia lignaria*) is a solitary, cavity-nesting species used for pollinating spring-blooming crops. Commercial stocks are sourced from a few locations in the western United States but are sold across the country. The existence of local adaptations of these bees are unknown, such as the propensity to nest in nearby provided materials or to disperse elsewhere. In spring 2019, California- and Utah-sourced blue orchard bees were deployed in cherry orchards in both states. Nesting sites were placed near (within 78 m) and far (500 m to 1 km) from central bee release points. Paint-marked bees were released when floral resources were available. Observations of marked bees at nesting sites were used to evaluate female retention and dispersal pattern. Nesting bee counts in March-blooming California orchards revealed a significant difference in female retention by origin; over twice as many UT bees established nests near the release site than did CA bees. Few females were found to have dispersed to far nesting sites. In May-blooming Utah orchards, counts of CA and UT bees were similar at near and far nesting sites; neither female retention nor dispersal were significantly affected by bee origin. That CA females were less likely to be retained in California orchards is concerning because the demand for commercial pollination is high for early-blooming California almond and cherry. Our results highlight the need to understand potential consequences of bee origin and their management on pollinator performance and

reproduction in target crops.

Introduction

Pollination of wild and domesticated plants is a key ecosystem service and is essential for growing the food that nourishes human populations. As pollen-collecting specialists, the life cycles of bees (charismatic insects belonging to the clade Anthophila [Hymenoptera: Apoidea]) are intimately tied to flowering plants; aspects of their anatomy, physiology, and behavior make them excellent pollinators. Globally, the cultivation and production of pollinator-dependent crops, and thus the demand for pollination services, has increased over the last several decades (Aizen et al. 2008, Aizen and Harder 2009, Calderone 2012).

Historically, much of the world has relied upon a single bee species, the western honey bee (*Apis mellifera* Linnaeus), to help produce most of its pollinator-dependent crops (McGregor 1976). However, honey bee populations in the U.S. have declined over the past several decades and costs to rent colonies for commercial crop pollination have soared, particularly for use in almond orchards (Aizen and Harder 2009, vanEngelsdorp and Meixner 2010, Bond et al. 2014, Kulhanket et al. 2017). Reliance on a single bee species as the sole crop pollinator is risky from both economic and environmental perspectives.

Of particular concern is the availability of crop pollinators for early blooming tree fruit and nut crops, which are high-value, pollinator-dependent sectors of agricultural production (Abrol 2015, Reilly et al. 2020). Pollination of tree crops, especially those in the family Rosaceae, require special attention due the large size of individual plants, high bearing capacities, self-incompatible pollen, frequent inclement spring weather conditions, and early bloom timing (Abrol 2015). For food security, additional (non-*Apis*) pollinators are needed to sustain economic and environmentally viable crop production. In addition to providing habitat for wild bees, one strategy to assure sustainable yields of pollinator-dependent crops is to employ alternate bee species that are managed for commercial use (Isaacs et al. 2017).

The blue orchard bee (*Osmia lignaria* Say) is a solitary, cavity-nesting bee that has increasingly been employed as a managed pollinator of commercial orchard crops. It is an excellent native pollinator of almond, apple, cherry, strawberry, and raspberry (Bosch et al. 2006, Artz et al. 2013, Sheffield 2014, Andrikopoulos and Cane 2018, Horth and Campbell 2018, Pitts-Singer et al. 2018). Blue orchard bees readily nests in artificial materials, prefers to visit blossoms of rosaceous fruit trees and berry bushes, and are naturally active in spring (Bosch and Kemp 2001). Field trials have repeatedly demonstrated the pollination efficacy of blue orchard bees on various crops and sometimes a positive return on bee population within orchards (Torchio 1981a, 1985, Bosch and Kemp 2002, Abel et al. 2003, Torchio 2003, Bosch et al. 2006, Boyle and Pitts-Singer 2017, 2019, Andrikopoulos and Cane 2018, Pitts-Singer et al. 2018). These features make the blue orchard bee well suited for management in early season commercial orchards.

As a solitary species whose management protocols are still being refined, scaling blue orchard bee production for commercial pollination to levels that can compete with honey bee services is challenging. Ongoing research on this species is continuing to inform management practices that help reduce population loss, and thereby increase sustainability of bee populations in orchard and propagation environments. Three main factors were identified by Bosch and Kemp (2001) in limiting population growth of blue orchard bees in commercial orchards: pesticide sprays, developmental and winter mortality, and pre-nesting female dispersal. Dispersal is a characteristic of any solitary, gregarious bee species and is the greatest limiting factor to sustaining their populations for agricultural production (Stephen 2003, Pitts-Singer 2013). High pre-nesting female dispersal inherently limits the number of bees that are retained within an orchard, and subsequently, the number of progeny produced for pollination the following spring.

Management practices that have been shown to affect pre-nesting dispersal, or conversely, retention of blue orchard bee females released in orchard environments are release method and nesting site distribution. Greater dispersal occurs when females are deployed as adults or ready-to-emerge cocooned adults that have been removed from nesting cavities compared to when cocooned bees remain inside their natal nests and emerge naturally (Bosch and Kemp 2001, Torchio 2003). However, the current industry practice to remove cocooned adults from nesting materials (i.e., loose cell system) is necessary to control pests and diseases, decrease storage space, and assess stock numbers. Therefore, to account for mortality and pre-nesting female dispersal, stocking rates in orchards are nearly twice as high as the number of bees likely needed for pollination, which can dramatically increase the cost of pollination services.

Inserting natal nests into cleaned nesting cavities along with sanitized loose cocoons is a strategy that combines the two release methods and has resulted in female retention rates comparable with releasing all bees in natal nests (Torchio 2003). Additionally, releasing a small batch of bees during early crop bloom and a large batch soon afterwards (known as batch-releasing or staggered release) also may reduce pre-nesting female dispersal because females in the later batch are attracted to the activity of the bees that have already established nests (Bosch and Kemp 2001, Torchio 2003). But these strategies require an attentive bee manager and extra effort, which may become impractical for large-scale pollinator deployment.

The style and arrangement of nesting sites within the orchard can also influence nesting distribution, female retention, and population return (Torchio 1981a, 1982a, Artz et al. 2013, 2014, Boyle and Pitts-Singer 2017). Early orchard studies found that pre-nesting blue orchard bees drift from release sites in all directions and to various distances (<100 m) where nesting sites are provided (Torchio 1981a,b, 1982a,b, 1984a,b). Artz et al. (2014) found that blue-colored nesting shelters were preferred over yellow- or orange-colored shelters, and that the number of bee release sites did not affect population return. Application of a chemical lure on nesting cavities makes them preferable over those without the lure, but whether the lure prevents blue orchard bees from dispersal to other sites is unknown (Pitts-Singer et al. 2016).

Other orchard studies have demonstrated that various measures of nesting success (e.g., female retention, population return, nest occupancy) is improved when nesting sites are distributed throughout an orchard rather than at a central location (Artz et al. 2013, Boyle and Pitts-Singer 2017). While nesting sites within orchards needs to be practical to implement, placement should be informed by female foraging range (roughly 100 m in *Osmia* spp.) to achieve uniform pollination (Vicens and Bosch 2000, Bosch and Kemp 2001, Boyle et al. 2020).

Blue orchard bees sold for commercial pollination are largely trapped and propagated from wild populations in the western United States because on-crop reproduction usually results in a net reduction without the availability of supplemental forage. Utah populations have been the most widely studied and employed as pollinators, but bees trapped and propagated in Washington, Oregon, Idaho, and California are also being used. Inasmuch, one constraint to having widespread commercial use of blue orchard bees is that populations from different climates exhibit regional variation in developmental phenology (Sgolastra et al. 2012, Pitts-Singer et al. 2014).

For instance, bees sourced from California have a longer summer aestivation as prepupae (cocooned 5th stadium larvae) compared to bees native to northern Utah and western Washington, presumably due to extended warm temperatures during the growing season (Bosch et al. 2000, Pitts-Singer et al. 2014). Thus, blue orchard bee populations seem to be regionally adapted such that their phenology matches their climate of origin, and bee management should be adjusted based on the population source and the climate in which they are used. Little research has investigated how geographically distinct populations may differ in other ways that influence their management, such as tolerance for dense nesting aggregations or propensity for pre-nesting dispersal.

For this study, we hypothesized that geographically distinct populations of blue orchard bees would differ in their retention near release sites, dispersal to distant nesting sites, and reproductive success. We predicted that females have locally adapted life histories or physiological traits that would result in better retention in orchards located within their natal ranges and respective bloom periods compared to females sourced from a non-natal region. Therefore, our main objectives were to determine the effect of natal origin on 1) female retention, 2) dispersal distance and direction, 3) population return in UT orchards. To meet these objectives, we used blue orchard bees sourced from

California and Utah and deployed them as pollinators in commercial cherry orchards in each state.

Materials and Methods

Bee source. Blue orchard bees were obtained from commercial suppliers, Foothill Bee Ranch in Foresthill, CA and Mountain West Mason Bees, LLC in Riverton, UT. In spring 2018, nesting materials were deployed for harvesting blue orchard bee progeny from wildland populations in two climatically distinct regions: the foothills of the Sierra Nevada Mountain range in California (CA bees) and the Rocky Mountain range in southern Idaho and northern Utah (UT bees). The CA bees typically emerge and nest in March whereas the UT bee populations are typically active during May.

Immature bees were managed by the suppliers. After reaching adulthood, bees were placed into cold storage for winter diapause on a schedule that would prepare them for use in their respective regions. Cocooned (diapausing) adult bees remained in cold storage until spring 2019. A total of 5,400 females and 9,000 males were used in the study, half from each population source. The purpose of using progeny from wild populations was to ensure that immature developmental phenology was consistent with their geographic origin through the adult stage and that any regionally adapted traits were expressed during field trials.

Study sites. In major cherry-growing regions of California and Utah, three conventionally managed commercial cherry orchards were selected for evenness in age and variety (6 orchards total) and separation distance (at least 2 km apart). In California, sweet cherry (*Prunus avium* Linnaeus ‘Bing’) orchards were located in San Joaquin County. Trees were 9 to 16 years old, intermixed with several pollinizer varieties, and grafted on Mahaleb cherry (*Prunus mahaleb* Linnaeus) root stock. Surrounding land use was dominated by commercial sweet cherry and walnut orchards, intermixed with urban development; non-crop floral resources and natural vegetation were sparse within and surrounding the orchards (personal observation).

In Utah, tart cherry orchards (*Prunus cerasus* Linnaeus ‘Montmorency’) were located in Utah County. Trees were 15 to 27 years old, of a single variety, and grafted on Mahaleb cherry root stock. The surrounding land use was dominated by commercial tart cherry orchards, intermixed with suburban development and wildlands; non-crop floral resources and other natural vegetation were plentiful in and around orchards (personal field observation).

Within each selected orchard, a 1.2-ha (110 m × 110 m) center section served as the target pollination area, where female retention (the proportion of females nesting per the number released) was measured. Within each center orchard section, 16 nesting sites (an aggregate of nesting cavities suitable for blue orchard bees housed in a nesting shelter) were evenly distributed in a 4 × 4 grid (Fig. 1-1). Shelters were constructed of dark blue corrugated plastic (length by width by height = 22 × 20 × 26 cm) (as in Artz et al. 2014).

Each nesting site contained 140 nest cavities constructed from cardboard tubes with paper straw inserts and glued-in plastic plugs at the back, zip-tied in bundles to the top of the shelter. All nesting cavities were 15 cm in length, with a diameter of 7.5 mm (100 cavities) or 8 mm (40 cavities). Shelters were secured with zip ties to a primary branch near the tree trunk at approximately 1-1.5 m in height and with the front facing southeast. Prior to bee release, nesting cavities were sprayed with a bee attractant at the recommended dose rate of 100 cocoon-equivalents (Pitts-Singer et al. 2016). A black polycarbonate “emergence shelter” was placed on a plastic stand approximately 30 cm above ground in the middle of each center section, which served as a central bee release point.

Beyond the center orchard section, 16 additional (distant) nesting sites were evenly placed in a concentric circle each at far (500 m) and very far (1 km in California or 750 m in Utah) away from the release point, for a planned total of 48 nesting sites per study site (Fig. 1-2). Distant nesting sites were used to measure female dispersal distance and direction from the release point. Due to variance in land ownership and unforeseen circumstances, the deployment of some distant nesting sites according to the experimental design was infeasible.

In California, there were missing nesting sites at Site 1 (four very far), Site 2 (three very far), and Site 3 (one far, two very far) for a total of 134 nesting sites across all three study sites. In Utah, there were missing boxes at Site 1 (one far and three very far) , Site 2 (two very far), and Site 3 (one very far) for a total of 137 nesting sites across all three study sites. ArcMap 10.7.1 was used to generate shapefiles with the positions of each nesting site. Shapefiles were then uploaded to ArcGIS Online and ArcGIS Collector phone application was used to navigate to the planned location for nesting site installation, bee observations, and nest retrieval.

Orchard bloom and weather conditions. It is desirable to release bees when 5-15% of crop flowers are open and favorable weather prevails to ensure ample forage for nesting and suitable flight conditions. Therefore, we targeted release for this estimate and measured orchard floral availability at center sections upon bee release and subsequent nest observation events (first and second in California, and second in Utah) (Table 1-1).

Four flowering branches were randomly selected on each of 20 cherry trees. On each selected branch, a 0.3 m section (approx.) was flagged, and every flower bud within the section was counted and categorized into one of four stages (green bud, white bud, open bloom, and post-bloom). The proportion of the total flowers that were in the open stage were used to document the availability of orchard flowers at center orchard sections throughout the experiment.

Bee release aligned with the targeted 5-15% bloom in orchard sites 2 and 3 in California, and exceeded those levels (but before peak bloom) at all other orchard sites. In California orchard sites, crop bloom was most abundant during the first bee observation event; bloom had dramatically decreased by the second bee observation and event and very few to no open flowers were available >20 days after bees were released (personal field observation). In Utah orchards, crop bloom was abundant and persistent from bee release through the second bee observation event; most flowers were post-bloom by the last bee observation event (personal field observation).

Weather conditions in both states were typical during our experiments. In California orchards, mean daily weather conditions for the duration of our experiment averaged 15°C, 2.9 m/sec windspeed, and 0.2 cm precipitation (Global Wind Altas 3.0, Weather Underground). Compared to mean historic values (16°C, 1.9 m/sec, 3.2 cm), weather conditions were cooler, windier, and drier (30-year average from the period 1991-2020, PRISM Climate Group). In Utah orchards, mean daily weather conditions during our experiment were 12°C, 3.2 m/sec windspeed, and 0.1 cm precipitation. Compared to mean historic values (11°C, 1.7 m/sec, 3.1 cm), weather conditions were warmer, windier, and drier.

Bee marking and release. To differentiate female bees by natal origin, bees were temporarily removed from cold storage, excised from cocoons, and marked with enamel paint on the dorsum of the thorax. In California orchards, bees from each source population and those released at each orchard site were painted a different color (total of six colors used). In Utah orchards, bees from the same population source were painted the same color at Sites 1 and 3 but were painted a different color at Site 2 (total of four colors used). Unique paint colors would inform whether bees dispersed between orchard sites; it was due to a miscommunication between field and laboratory personnel that bees from each source were not uniquely paint-marked at all three Utah orchard sites.

After marking, females were placed back in cold storage (3°C to 6°C) until deployment. In California, female bees at Sites 1 and 2 were held in cold storage after marking for one to three days before they were released (Table 1-2). Due to a delay in orchard bloom, female and male bees for Site 3 were held for seven to eight days after marking until released. In Utah orchards, females were held in cold storage after marking for one day before release at Sites 1 and 2, and two days at Site 3.

In both states, male cocoons were incubated at room temperature until emergence reached approximately 50%, and then were held in cold storage until released, 5 to 12 days later. Females found to have emerged from supposed male cocoons were removed from the study because they were unmarked. However, because some bees designated as males were still cocooned upon release, it is possible that some unmarked females could have been released with our male population.

During transport (approx. 90-140 min), bees were stored in vented plastic containers inside a Styrofoam cooler without a cooling pack, so that bees gradually warmed to ambient temperature prior to release. At each center orchard section, 900 painted females and 1,500 males were released by gently shaking adult bees and cocoons into each emergence box. Half of the bees released at each site were from each population (CA or UT). Bees that did not immediately fly upon release could later exit emergence shelters through a downwards spout at the bottom of the box.

Bees were released in California orchards on 21 March (Sites 1 and 2) and 28 March (Site 3), and in Utah orchards on 25 April (Site 1) and 28 April (Sites 2 and 3) (Table 1-2). Bees were preferentially released between 0900 and 1100 hours, and on days conducive to bee flight and nesting activity (temperatures >12°C, windspeeds <21 kph, and little to no precipitation) as it has been suggested that rain and wind may encourage pre-nesting dispersal (Bosch and Kemp 2001). Upon release, bees were observed mating on and around emergence shelters and foraging on orchard flowers. In addition to the blue orchard bees we released, orchards were stocked with honey bee colonies at the full rate, five colonies per ha (two colonies per ac) in California and two colonies per ha (0.75 colonies per ac) in Utah.

Nesting site evaluations. Three times during the pollination season, we evaluated nesting activity at all nesting sites and at all orchard sites (except Site 1 in Utah, which was only sampled twice) (Table 1-2). In California orchards, nesting bee observations were made at 3-9 days, 14-20 days, and 20-28 days after bee release. In Utah orchards, observations were made 5-7 days, 11-14 days, and 18 days after bee release. Observations preferentially occurred on days when weather was expected to be favorable for bee flight (temperatures >12°C, windspeeds <6 m/sec, and little to no precipitation).

All nesting sites at a given orchard site were observed on the same day between 1000-1700 hours. Center nesting sites were observed for 10 min each in California and 15 min each in Utah orchards. In both locations, distant nesting sites were observed for 5 min each, unless a bee or evidence of nesting activity was detected (loose pollen, cavities plugged with soil, etc.), in which case, the nest box was observed for 10 min. Distant nesting sites were observed for a shorter period of time due to a constraint in personnel and the need to observe all 48 nesting sites on the same day and during bee foraging hours. During nest observations, any female observed was recorded along with her behavior and paint color or absence of paint.

A female bee was recorded as nesting if she displayed one or more of the following behaviors: occupying a single nesting cavity for one minute or more, occupying and guarding a single cavity from other females, flying into a cavity with pollen or mud, building a mud wall, or backing out of a cavity with pollen on her scopa and then spinning around to reenter rear-first in order to deposit the pollen load. Other behaviors, such as “sunning” (perching on the nesting materials to bask in the sun), “sheltering” (remaining inside of a nesting cavity), and “browsing” (flying in and out of several nesting cavities) were also recorded. To assure reliable counts of nesting females and reduce the chance of re-counting the same individual, only observations that were categorized as nesting were used for statistical analyses. Nesting behaviors accounted for 67.8% of all recorded observations in California orchards and 84.2% in Utah orchards.

The California and Utah orchard trials were treated as separate experiments, and the data were independently analyzed. To compare female retention as an effect of bee origin, the data was pooled across observation events for each nesting site and then summed the count of nesting females at 1) center nesting sites and 2) distant nesting sites. These data were analyzed by state of origin using a generalized linear mixed model with a zero inflated Poisson distribution, with nesting site and orchard site (3 in CA and 2 in UT) as random variables and with nesting site nested within orchard site. A zero inflated Poisson distribution was used because the count data had a substantial amount of

Analyses were run using R 4.0.0 and packages glmmTBM (Brooks et al. 2017) and mgcv (Woods et al. 2016). We used the same model to evaluate the effect of bee origin on female dispersal in Utah orchards, using the count of nesting females at distant nesting sites. Additionally, we ran a combined model, including both center and distant nesting site observations. Since combined model and singular observation models generated the same results, the singular observation models are reported (Table 1-3).

Additionally, during observations in Utah, we marked each nesting cavity containing a nesting female with a permanent marker to determine our detection probability (the proportion of nesting cavities containing bee progeny in which we observed a female exhibiting nesting behaviors out of all the nesting cavities later found to contain bee progeny). After crop bloom and nesting ceased, nesting sites from Utah orchards were photographed and systematically disassembled in the laboratory. Nesting cavities were left intact, stored at outdoor temperatures in a sheltered, screened environment during summer (for continuation of bee development to adult eclosion, and then moved to cold storage (4°C) in October 2019 for the duration of winter.

The number of cavities containing bee nests was determined from visual inspection and was used in calculation of the nesting female detection probability. Bee population return (a percentage determined by dividing the number of females produced by the number of females released ×100) was calculated for each orchard site from the number of adult cocooned bees within nests and anticipated sex based on size and brood cell position, using radiographic image diagnosis (12-second exposure at 24 kVp; Faxitron 43804N, Faxitron Bioptics, Tucson, AZ). Images were taken in November 2019 so that offspring development to the adult stage could be documented and mortality at other life stages could be assessed. Similar reproduction data for nests produced in California orchard sites were not collected due to a lack of time, personnel, and appropriate facilities.

Results

Female retention. In California sweet cherry orchards, analysis of nesting bee counts at the center orchard sites revealed a significant difference in female retention by geographic origin (F = 2.478, df = 280, P = 0.0132); UT females (n = 105 bees; bees per nesting site mean ± SE = 2.19 ± 0.30) accounted for over twice the number of nesting bees than CA females (n = 43; 0.90 ± 0.19) (Fig. 1-3A). Across all three observation events, nesting bee counts captured 5.5% of the female population released, most of which was detected during the first observation event (Fig. 1-3B). Heat maps of marked females at center nesting sites show the increased number of UT bees compared to CA bees but did not reveal any visible directional preference or nesting pattern (Fig. 1-3C).

Contrary to results in California orchards, bee origin did not have a significant effect on female retention in later-blooming Utah tart cherry orchards (F = -0.082, df = 248, P = 0.935), as counts of nesting CA bees (n bees = 233; bees per nest box mean ± SE = 4.85 ± 0.54) and UT bees (n = 264; 5.50 ± 0.54) at center nesting sites were similar (Fig. 1-4A). Across all observation events, nesting bee counts captured 18.4% of the released female population, most of which was detected during the second observation event (Fig. 1-4B; Site 1 bee nesting was not observed a third time). Heat maps of nesting females at center orchard sections show similar counts of nesting by bee origin, and that bees nested in all directions from the central release point (Fig. 1-4C).

Female dispersal. In California orchards, five CA bees and six UT bees were counted at distant nesting sites (500 m to 1 km from the release point) across all three observation events. As a consequence, our sample size was too low to statistically analyze the effect of geographic origin on female dispersal. In Utah orchards, the mean number of CA females (n = 32; mean bees ± SE = 0.33 ± 0.10) and UT females (n = 45; 0.46 ± 0.09) at distant nesting sites (500 m to 750 m) demonstrated that bee origin did not have a significant effect on female dispersal (F = 0.031, df = 248, P = 0.9755). The furthest dispersal distance observed was approximately 2.7 km, when marked bees from Site 2 were found at Site 3 and vice-a-versa. Heat maps of nesting marked females at distant nesting sites showed no directional preference in nesting between sites (Fig 1-5).

Nesting female detection. In Utah orchards, the nesting female was observed for approx. 53% of the occupied cavities (contained a nest with at least one brood cell) at center nesting sites. At distant nesting sites, approx. 43% of occupied cavities were captured by our field observations. Thus, the true nesting population was as much as twice the nesting bee counts made from our observations, each female observed could have made brood cells in approximately two nest cavities, or some combination thereof.

Unmarked nesting females. In California orchards, unmarked females were detected nesting in our provided materials at center orchard sites (n = 13) but not at distant nesting sites. In Utah orchards, unmarked bees were found nesting at center orchard sites (n = 206) and at distant sites (n = 208; evenly split between nesting sites 500 m and 750 m from the bee release point).

Bee population return. Of the 2,700 females and 4,500 males released at all three center orchard sections (48 nesting sites) in Utah, a total of 1,212 cocooned adult females and 3,135 cocooned adult males were recovered as progeny, a 60% return on the bee population (range = 47-82%) and a 45% return on the female population (range = 31-61%). Across entire study sites (including distant nesting sites), a total of 2,142 adult females and 4,853 adult males were recovered, meaning a 97% return on the bee population (range = 59-134%) and a 79% return on the female population (range = 44-115%).

From all the nesting cavities we provided in Utah orchards, a total of 1,533 blue orchard bee nests contained 8,022 individual brood cells. The proportion of brood cells that succeeded to the cocooned adult stage was 87%. The greatest single cause of in-nest mortality was due to pollen balls (no egg was laid on the food provision or the bee died in the egg stage). Dead larvae and pupae were found at a lower rate, and population loss due to pests and disease was minimal. The mean sex ratio across all three center orchard sites was 2.6 males to one female. A higher proportion of female progeny was observed at distant nesting sites than at center nesting sites (mean sex ratio of 1.9 males to one female). In California orchards, nesting sites were not systematically disassembled, and so, population return was not calculated.

Discussion

Our experiments demonstrated that blue orchard bee origin has a significant effect on female retention (post-release nest establishment) under certain contexts. In early-blooming California sweet cherry orchards, we were surprised to find that CA females established nests at a significantly lower rate than did UT females, as we expected bees natal to the orchard region to perform best. Bees of both geographic origins performed better in Utah than in California cherry orchards; more nests were established at center and distant nesting sites. In these later-blooming Utah tart cherry orchards, bee origin did not have a significant effect on female retention. The varying response in nest establishment due to geographic origin of blue orchard bees released in California and Utah orchards highlight the importance of regional adaptations on performance given the crop, and thus location and timing of their deployment, in which they are utilized as pollinators.

Bee behavior is multidimensional and several other factors, in addition to geographic origin, could have influenced nest establishment. For example, CA and UT bees were exposed to different conditions during larval development, summer prepupal quiescence, storage during fall, and winter diapause. Since the life stage duration of these wild-sourced bees were unknown until managed as cocooned adults, we can only presume that the CA bees were slower to reach adult eclosion than the UT bees as has been found previously (Bosch et al. 2000, Pitts-Singer et al. 2014). Therefore, depending on the timing of management (via temperature control) prior to placing them at a constant, cold wintering temperature (early December for CA bees and mid-November for UT bees), the adults may have experienced different durations of wintering temperatures since fall and early winter are cooler in UT than in CA.

Due to the timing of orchard bloom, bees from both stocks had been exposed to a shorter wintering period when released in California orchards than in Utah orchards, by approximately one month. It is important to recognize differences in wintering duration (and thereby, age of adult bees, maturity of ovaries, and amounts of metabolic reserves) prior to release, as previous research has found a positive effect of sufficiently long, cold wintering periods on post-emergence performance (Bosch and Kemp 2003, Sgolastra et al. 2010, 2016). If CA bees had a shorter duration of experience cold temperatures than did the UT bees, then performance (nest establishment and/or survival) of CA females could have been negatively impacted by a shorter wintering duration upon release in California orchards.

A number of other factors could have variably affected bee performance and/or our ability to detect them. For example, bee size can influence foraging range and homing ability such that larger bees tend to cover greater distances than smaller bees (Guédot et al. 2009, Greenleaf et al. 2007). From this research, I have repeatedly observed that CA females are smaller than UT females (personal observation, Chapter III this thesis); observations here could be skewed such that smaller CA bees had better detection due to their shorter, more frequent trips to their nest while foraging. Varying preferences for nesting material and/or dimensions, floral resources, and soil composition could have also varied between these geographically distinct blue orchard bee populations, and thus, impacted their survival and performance in our experiment, but was out of the scope of this project.

In addition to evaluating the retention and dispersal of female bees we released, we also detected immigration of unmarked (resident wild or locally managed) bees into the orchard. In California orchards, the small number of unmarked females we detected were only at the center nesting sites, suggesting that they were among the small cocoons designated as male, rather than from local bee populations. In Utah orchards, unmarked females were abundant at center and distant nesting sites, which suggests that these unmarked bees were from local populations, wild and/or managed. No other managed blue orchard bee populations were released within our experimental sites, although growers nearby could have had managed populations. Nonetheless, prevalent unmarked bees in Utah orchards demonstrated that blue orchard bees readily immigrate to commercial orchard crops when suitable nesting cavities are available.

Despite apparently low female retention in Utah orchards (nesting bee counts captured 18.4% of the female population released), we found relatively high mean population return within center nesting sites (approx. 60%) and entire orchard sites (approx. 97%). Given that 29% of all the nesting bee counts at site centers and 74% of all the nesting bee counts at distant nest boxes were of unmarked bees, the bee population returned cannot be attributed only to the (marked) bees we released. Due to the gregarious nesting habit of blue orchard bees, the abundance of unmarked bees in Utah orchards may have contributed to the enhanced nest establishment of the marked bees we released (Torchio 1976).

The frequent female occupancy of nesting cavities at far distances (between 500 m to 750 m) in Utah orchards, in addition to those cavities near (within 110 m) the bee release site, accounted for the replacement of an additional 37% of the bee population, or 34% of the released female population. While the strategy we employed (deploying numerous nesting sites in concentric rings outside of the target pollination area) may not be practical to implement on a large scale, providing additional nesting substrates for bees at orchard perimeters or neighboring orchards could be a strategy for maximizing pollination services and bee population return.

Other studies investigating blue orchard bee performance in orchard environments have primarily used population return or counts of females occupying nesting cavities at night as measures of establishment and reproduction (Torchio 1982a, Bosch et al. 2006, Artz et al. 2013, 2014, Boyle and Pitts-Singer 2017, 2019, Pitts-Singer et al. 2018). However, in these studies, bees released by researchers usually were not marked to differentiate them from local bees, even in locations where blue orchard bees naturally occur during orchard bloom. Without knowing if the number of bees and nests is attributed only to the bees intentionally added to the orchard, the resulting return on bee population as offspring may not represent reproduction by the managed bees. Based on our findings of marked and unmarked bees in our experiments, it is likely that previous studies investigating blue orchard bee reproduction in California orchards have reflected the output of the population released since local bees are likely absent, and that studies in Utah orchards were likely influenced by resident (managed or wild) populations nesting within or near commercial orchards.

Our study also revealed novel information about long-distance movement of blue orchard bees within commercial orchard settings. In both orchard regions, we detected marked females nesting at the furthest distance tested. In California orchards, blue orchard bees dispersed (and established nests) at least 1 km away from the point of release, although very few females were observed at these distant nesting sites. It is not clear whether dense agricultural landscapes such as those found in the Central Valley of California somehow impaired bee nesting migration or survival in general. In Utah orchards, the frequent occupancy of marked females at distant nesting sites (between 500 m and 750 m from the bee release point) demonstrated that blue orchard bees readily migrate within commercial orchard landscapes. The dispersal characteristic of the blue orchard bee was exemplified when bees were found to have dispersed (and established nests) 2.66 km away from the orchard of release to another, similar orchard. To our knowledge, this is the furthest documented dispersal distance of blue orchard bees within a commercial orchard landscape.

Our plots of the density of nesting bees at distant nesting sites show no pattern of nest establishment that may indicate the influence of an environmental factor (e.g., sun rise, sun set, prevailing wind, pesticide application) or attraction of a landmark (e.g., mountains, pasture) or other flowering crop. Within site centers, heat maps also revealed no pattern for the location of certain hot spots of nesting. Bees nested in all directions and at varying distances from a central release point, in agreement with previous research (Torchio 1981a, 1982b, 1984b). More data about those sites of high nesting activity might have been revealed if exposure to such variables as sunlight, shade, or an area of damp soil for nest-building may have made certain nesting sites preferable to others.

Reducing pre-nesting female dispersal, and thereby enhancing nest establishment and population return, remains a major challenge for sustaining managed blue orchard bee populations in large commercial orchards. High return on bee populations not only reduces or negates the purchasing of bees each year but also helps to alleviate the current need to trap bees from wild populations. We directly measured female retention in commercial orchards by identifying (i.e., marking) individuals and searching for them at nesting sites. The significant difference in female retention due to bee origin that we observed in California orchards are particularly meaningful for commercially managed populations, as the demand for blue orchard bee pollination services is greatest in early blooming California fruit crops such as almond and cherry.

Our results suggest that careful attention to the geographic origin and management history, particularly concerning wintering practices, is needed when using blue orchard bees in early blooming crops. The current practice of trapping and transporting blue orchard bees from regions with different climates than from where they are used as pollinators will continue unless local bees can be propagated reliably. It would be advantageous for future research to investigate whether geographically disparate populations may have variable preferences for nesting material and dimensions, floral resources, and other factors that may impact their performance in agricultural environments. Identifying distinct behavioral adaptations that could be useful for blue orchard bee management in various crops or growing regions would reinforce the need to limit movement (via policy or otherwise) of blue orchard bee populations between climatically distinct geographic regions.

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Tables

Table 1-1

Orchard Floral Availability

Event	**CA Orchards**			**UT Orchards**		
	Site 1	**Site 2**	**Site 3**	**Site 1**	**Site 2**	**Site 3**
Bee Release	2,264 (48%)	301 (8%)	586 (13%)	2,204 (25%)	1,714 (41%)	2,065 (52%)
Bee Observation 1	2,205 (51%)	1,122 (39%)	1,256 (39%)	NA	NA	NA
Bee Observation 2	0 (0%)	351 (16%)	351 (16%)	958 (13%)	1,416 (35%)	968 (23%)

Note. Units are the count and estimated percent of flowers in the open bloom stage at bee release and the first two nesting bee observation events (flower counts were not made during bee observation event 1 in Utah orchards) in center orchard sections in California and Utah. Very few to no flowers were present during observation event 3 (personal field observation), and thus, orchards were not surveyed in either state.

Table 1-2

Timeline of Experimental Events

Date	Orchard Location	Event
18 March	California	Paint marked female bees for Site 1.
19 March	California	Marked half of females for Site 2.
20 March	California	Marked remaining females for Site 2 and half for Site 3.
21 March	California	Marked remaining half of females for Site 3. Released bees (450 ♀ and ~750 ♂ per bee origin per site) and assessed orchard floral availability at Sites 1 and 2.
28 March	California	Released bees at Site 3 and assessed orchard floral availability.
29 March	California	Nest observation 1 and floral assessment at Site 1.
30 March	California	Nest observation 1 and floral assessment at Site 2.
31 March	California	Nest observation 1 and floral assessment at Site 3.
09 April	California	Nest observation 2 and floral assessment at Site 1.
10 April	California	Nest observation 2 and floral assessment at Site 2.
11 April	California	Nest observation 2 and floral assessment at Site 3.
16 April	California	Nest observation 3 at Site 1.
17 April	California	Nest observation 3 at Site 3.
18 April	California	Nest observation 3 at Site 2.
24 April	Utah	Paint marked female bees at Site 1
25 April	Utah	Released bees at Site 1 and assessed floral availability.
26 April	Utah	Marked female bees for Site 3.
27 April	Utah	Marked female bees for Site 2.
28 April	Utah	Released bees at Sites 2 and 3 and assess floral availability.
02 May	Utah	Nest observation 1 at Site 1.
03 May	Utah	Nest observation 1 at Sites 2 and 3.
09 May	Utah	Nest observation 2 and floral assessment at Sites 1 and 3.
10 May	Utah	Nest observation 2 and floral assessment at Site 2.
16 May	Utah	Nest observation 3 at Sites 2 and 3. Site 1 nesting sites were not observed a third time, as orchard floral availability was sparse at all three center sections (personal field observation) and nesting activity was sparse at the distant sites.

Note. California- and Utah-sourced blue orchard bees (*Osmia lignaria*) were flown in both states during cherry bloom in spring 2019; in each state, three orchards were used for this study.

Table 1-3

Pooled Counts of Nesting Female Bees by Origin and Experimental Location

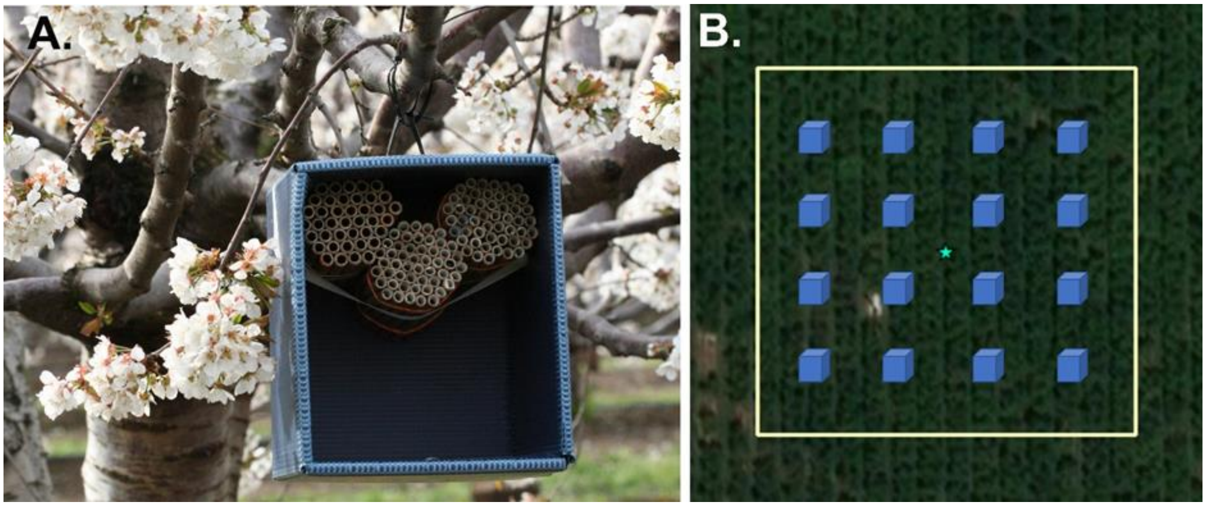
Location	Bee origin	Center sites	Distant sites	Total
CA	CA	43	4	47
CA	UT	105	7	112
UT	CA	233	32	265
UT	UT	264	45	309

Note. Bue orchard bees were sourced from California and Utah wildland populations and released in cherry orchards in both states in 2019. Data is pooled across all nesting sites and study sites by bee origin in each state.

Figures

Figure 1-1

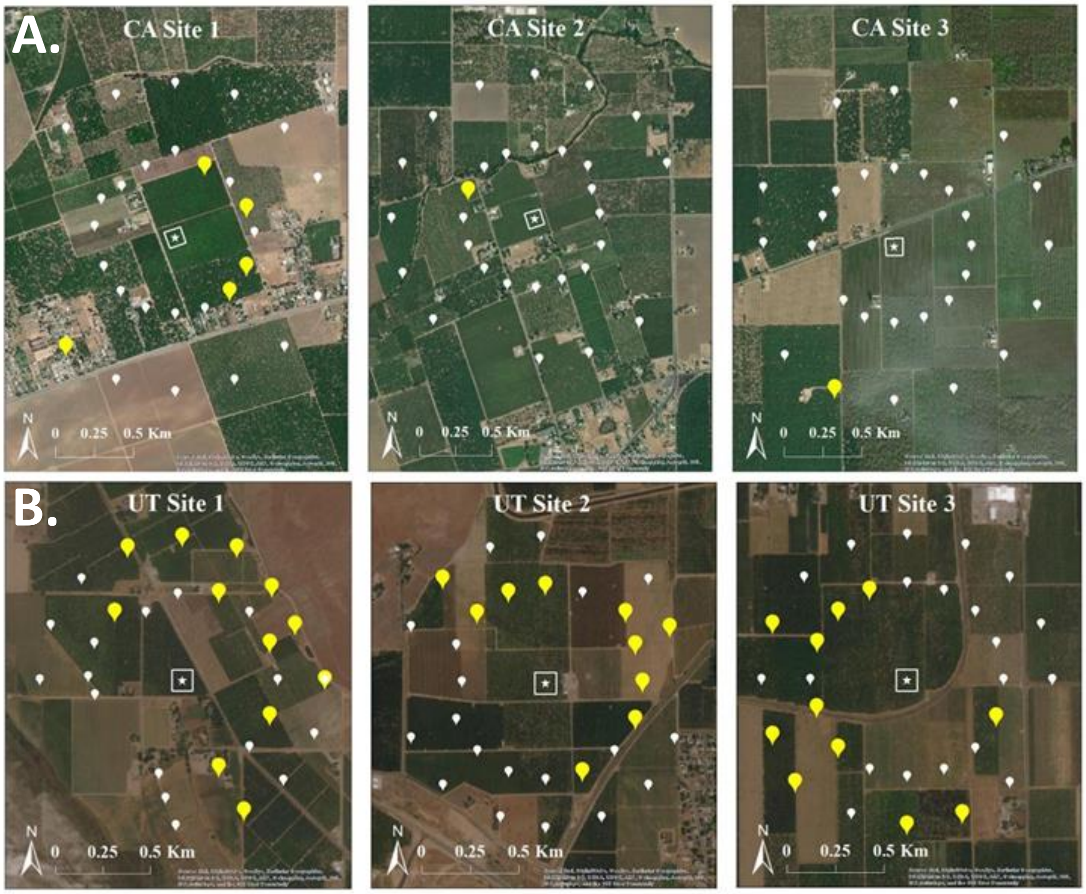
Nesting Materials and Layout of Nesting Sites Deployed in Center Orchard Sections



Note. (A) Each blue orchard bee nesting site was an aggregate of nesting cavities (bundled cardboard tubes with paper straw inserts) strapped to the upper interior of the shelter constructed of a blue corrugated plastic. (B) The layout of nesting sites deployed at center orchard sections, a 110 m × 110 m (1.2 ha) interior section of a commercial cherry orchard that is delineated by the yellow square. The star indicates the central position of the bee emergence shelter, and the blue cubes indicate the positions of center nesting sites.

Figure 1-2

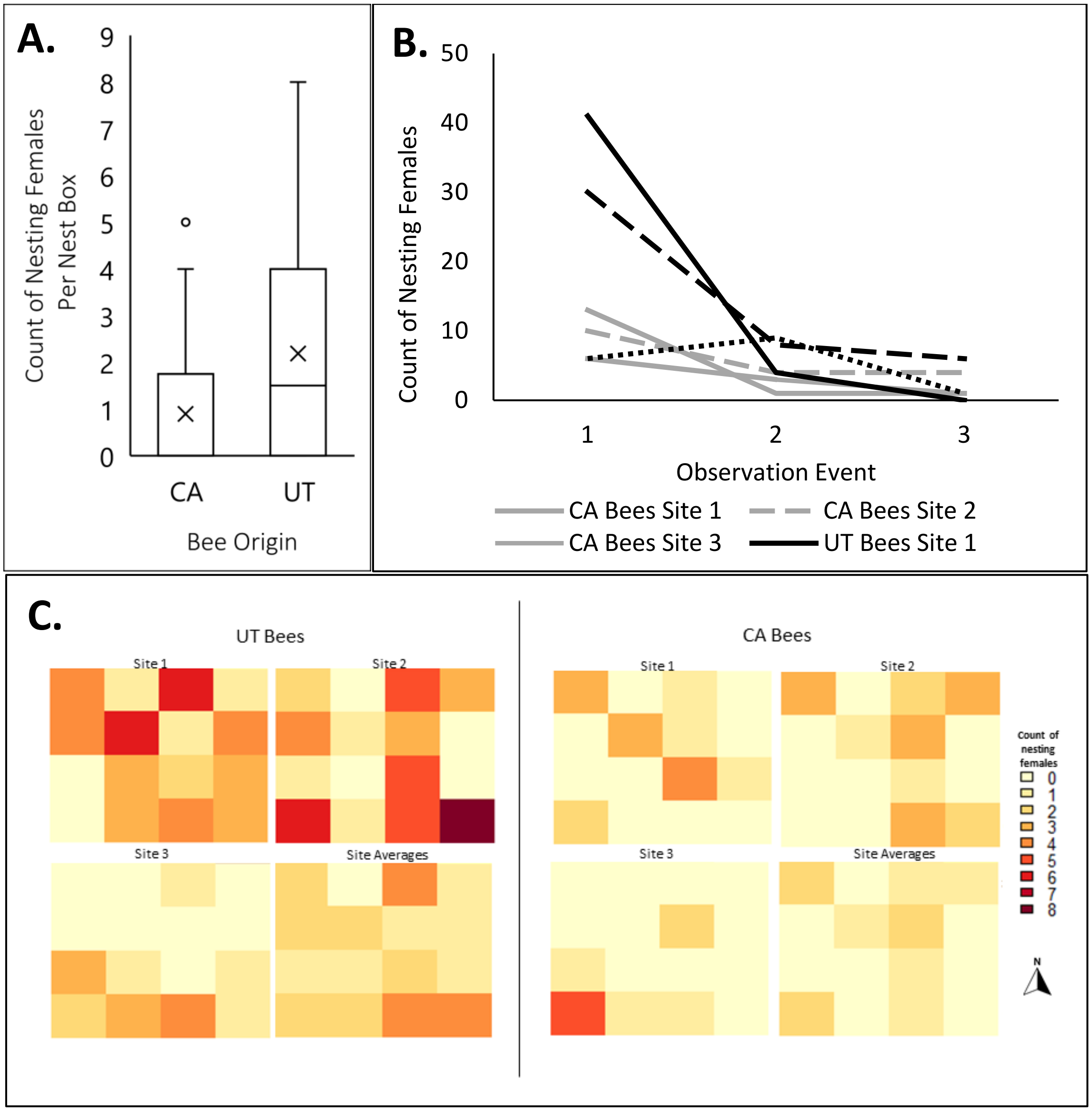
Site Maps in California and Utah Cherry Orchards



Note. Aerial view of (A) sweet cherry orchard study sites in San Joaquin County, California and (B) tart cherry orchard sites in Utah County, Utah. For each study site, the center white square delineates the 1.2 ha (3 acre) center section, where 16 blue orchard bee nesting sites were evenly distributed throughout; the star in the center represents the bee release point. The white and yellow pins (inverted drop-shaped icons) indicate nesting site locations at far (500 m, inner circle) and very far (1 km, outer circle) distances from the central release point. Nesting sites indicated by the larger, yellow pins are where one or more marked nesting females were detected.

Figure 1-3

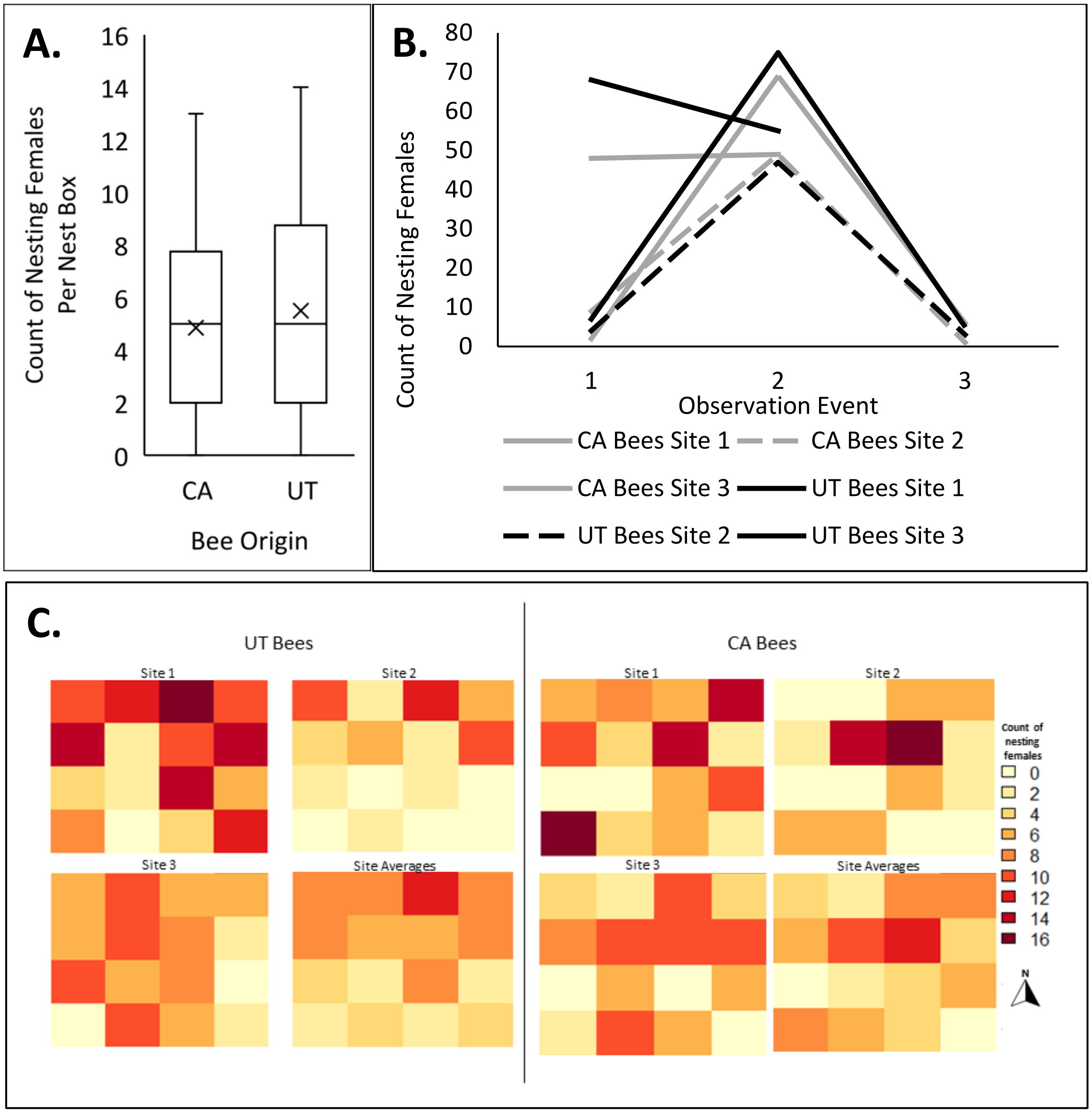
Female Retention and Nesting Density in California Orchards



Note. In California sweet cherry orchards in 2019, nest establishment (retention) by marked blue orchard bee females in center orchard sections deployed at three study sites. (A) Box plot showing minimum, first quartile, median, third quartile, maximum counts, and outliers of CA and UT bees retained at center nesting sites; the data was pooled across observation events for each nesting site by study site. (B) Counts of CA and UT bees during each of the three observation events; bees were released on 21 March (Sites 1 and 2) and 28 March (Site 3), and observations were made 3-9 days, 14-20 days, and 20-28 days after release. (C) The mean counts of CA and UT bees, with data pooled for all three observation events for each study site; each colored square represents one nesting site, and its color shows the count of nesting females.

Figure 1-4

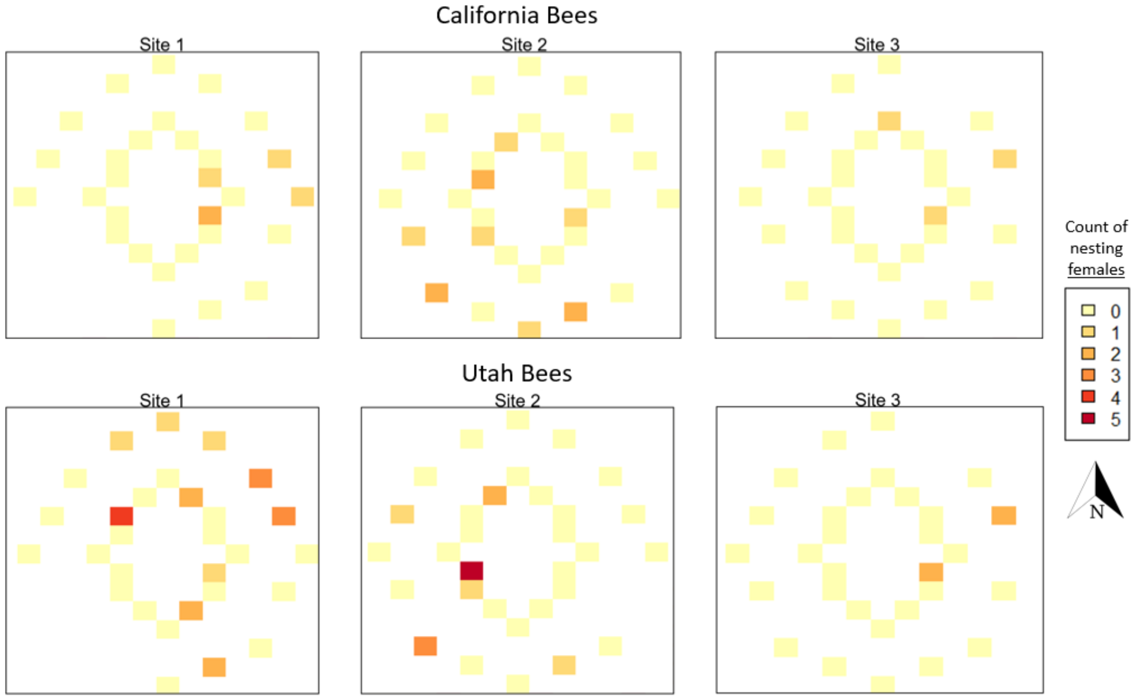
Female Retention and Nesting Density in Utah Orchards



Note. In Utah tart cherry orchards in 2019, nest establishment (retention) by marked blue orchard bee females in center orchard sections deployed at three study sites. (A) Box plot showing minimum, first quartile, median, third quartile, maximum counts, and outliers of CA and UT bees; the data was pooled across observation events for each nesting site by study site. (B) Counts of CA and UT bees during each of the three observation events; bees were released on 25 April (Site 1) and 28 April (Site 2 and 3), and observations were made 5-7 days, 11-14 days, and 18 days after release. (C) The mean counts of CA and UT bees, with data pooled for all three observation events for each study site; each colored square represents one nesting site, and its color shows the count of nesting females.

Figure 1-5

Nesting Density of Bees at Distant Nesting Sites in Utah Orchards



Note. Counts of CA and UT marked blue orchard bee females dispersed to nest 500 m (inner circle) and 750 m (outer circle) away from a central bee release point in Utah tart cherry orchards in 2019, with pooled data for all observation events for each study site. Each colored square represents one nesting box, and its color shows the count of nesting females. Missing values indicate nesting sites that were not deployed in the field.

CHAPTER II

EFFECTS OF GEOGRAPHIC ORIGIN AND TEMPERATURE ON BLUE ORCHARD BEE SURVIVAL, DEVELOPMENT, AND EMERGENCE

Abstract

The blue orchard bee (*Osmia lignaria*) is a widespread North American native pollinator that is commercially available for spring-blooming fruit crop production. Wildland populations in northern Utah have been the most widely studied and employed as pollinators, but bees from other locations are also being used commercially. Some differences in developmental phenology between geographically distinct populations have been documented, but little is known about how bees from various regions respond to recommended management practices or translocation within and between ecoregions. In spring 2019, blue orchard bees sourced from Utah and Washington were reared in laboratory incubators under two thermal regimens: 1) standard constant temperatures used to manage bees through development, wintering, and adult emergence, and 2) hourly fluctuating temperatures programmed to mimic the natural daily (24 h) thermal cycles of the nearest cherry orchard growing region through their entire life cycle. Bees reared at constant temperatures had increased survival and shortened egg-adult development periods compared to bees reared at natural temperatures; however, this effect was greater for UT bees than for WA bees. At natural temperatures, high mortality coincided with the hottest maximum temperatures and late life stages (prepupa-adult). Females from both populations emerged in synchrony with local crop bloom at natural temperatures, but the emergence period was prolonged compared to the constant thermal regimen. Our study supports that bees originating from montane habitats suffer from the

warmer climatic conditions of lower altitudes where crops are cultivated, even when sourced within the same geographic region. To maximize blue orchard bee survival and performance as commercial pollinators, exposure to extreme summer temperatures should be negated and stocks from distinct geographic locations should be kept as separate cohorts for management.

Introduction

Pollinators of wild and cultivated plants play a key role in sustaining diverse ecosystems and aiding in the production of food crops that nourish humanity. As pollen-collecting specialists, bees (charismatic insects belonging to the clade Anthophila [Hymenoptera: Apoidea]) are the only commercially managed animals for pollination services. Honey bees (*Apis* spp.) and bumble bees (*Bombus* spp.) are the most well-known managed pollinators, but other bees such as mason bees (*Osmia* spp.) and the intensively managed alfalfa leafcutting bee (*Megachile rotundata* F.) are also being utilized (Delaplane and Mayer 2000).

Bee management and commercialization vary by species. For honey bees, the typical scenario is that growers contract beekeepers each year for crop pollination; an agreement outlines the cost for a given quantity and quality of colonies and details the timeline and responsibilities of both parties (Ferrier et al. 2018). Bumble bee colonies are reared in commercial facilities and shipped, or otherwise transported, to growers for pollination services in field or enclosed crops (Velthuis and van Doorn 2006); these colonies are not managed for propagation, and therefore, must be purchased each year. Solitary, cavity-nesting bees require unique management practices, but with relatively little input compared to social bees. Mason and leafcutting bees are typically purchased and managed by the grower for pollination services year after year; because propagation can be limited in agricultural environments, a portion of bees may need to be purchased some years to replace a deficit in the managed population (Pitts-Singer and Cane 2011, Peterson and Artz 2014).

The blue orchard bee (*O. lignaria* Say) is North America's most agriculturally important native mason bee. The natural distribution of the blue orchard bee is vast, occurring throughout much of the United States and southern Canada. It is an effective pollinator of rosaceous fruit tree (e.g., almond, apple, and cherry) and berry crops (e.g., raspberry and strawberry) (Torchio 1985, 2003, Brittain et al. 2013, Bosch et al. 2006, Artz et al. 2013, Andrikopoulos and Cane 2018, Horth and Campbell 2018, Pitts-Singer et al. 2018). To date, managed blue orchard bee populations can not only meet the pollination requirements of the small orchardist, but also can provide growers a much-needed supplement to honey bees for large scale commercial orchard pollination, especially in early-blooming almond and cherry (Stephen 2003, Bosch et al. 2006, Peterson and Artz 2014, Pitts-Singer et al. 2018).

Blue orchard bees can be purchased from commercial mason bee suppliers, who may also provide consultation and assistance to growers on how to use and manage this pollinator on their farms. Reputable mason bee suppliers keep track of the natal origin of their bees, collected via trap nests or propagation, and provide growers with bees that are assumed to be suitable for their region. However, mason bees that are natal to a particular ecoregion where their services are desired may not be readily available, and so, bees are often shipped from one climatically distinct locality to another. Blue orchard bees translocated to orchard-growing regions often experience warmer temperatures than that of their natal locations, as they are naturally abundant in montane, riparian habitats (see methods).

One constraint to having a more widespread commercial use of blue orchard bees is that populations from different climates exhibit regional variation in physiological responses (Sgolastra et al. 2012, Pitts-Singer et al. 2014). For instance, in order to cope with the extended warm California summer, prepupae (cocooned 5th stadium larvae) of bees native to California have a longer summer diapause compared to bees native to Utah and Washington (Pitts-Singer et al. 2014). If bees from Utah and Washington produce offspring in California during February almond bloom, then those progenies are unlikely to survive if not managed under controlled temperatures for much of the year. High mortality occurs when bees become adults so early in the summer that they deplete their fat reserves before winter (Sgolastra et al. 2011, Pitts-Singer et al. 2014).

Thus, an understanding of the blue orchard bee life cycle, which depends to some extent on bee origin, is necessary for successful crop pollinator management. A detailed description of the blue orchard bee life cycle is given by Torchio (1989) and phenological differences among populations are discussed by Bosch et al. (2008). While the natural activity time of blue orchard bees overlaps with the bloom time of many orchard crops in their local environments, greater precision in timing adult activity with crop bloom can be manipulated through established protocols for cold storage and incubation (Bosch and Kemp 2000, Orchard Bee Association 2021). Additionally, temperature management can maximize survival and post-emergence performance (Bosch and Kemp 2003).

While there are some documented differences in blue orchard bee ecophysiology between populations (e.g., Sgolastra et al. 2012, Pitts-Singer et al. 2014), little is known about optimizing use and production of these bees from different regions so that their management is straightforward and practical. The vast majority of published research that has informed management practices has been performed on bees sourced from northern Utah and southern Idaho, where the bee was first developed as a managed pollinator. Now, the largest supplies are collected from wildlands in Utah and Washington, with lesser supplies coming from California, Idaho, Oregon, and Wyoming. A better understanding of blue orchard bee ecophysiology throughout its range will further the development of best management practices for making decisions about using bees from various ecoregions according to the place and season of crop bloom.

For our study, we were interested in the separate and interactive effects of geographic origin of blue orchard bees (trapped from wildlands) and the temperatures they experience during a life cycle in local growing regions when they are used as managed pollinators. We reared bees from Utah and Washington in laboratory incubators under two thermal regimens, 1) “constant” – one temperature through immature development, one for winter dormancy, and one for adult emergence, and 2) “natural” – hourly fluctuating temperatures programmed to mimic the natural daily (24 h) thermal cycles of the nearest cherry orchard growing region for the entire bee life cycle. The former treatment was a shared “common garden” in which bees from both populations experienced the same, managed temperature conditions during their spring-summer immature development, as cocooned adults in the fall and winter, and during induced adult emergence (for two temporally separate pollination events) in spring; this treatment allowed for a direct comparison of observed variables by origin only. The latter treatment was specific to bee origin and served to compare observed variables at fluctuating (natural orchard) temperatures to the same outcomes at constant temperatures for each bee population.

Materials and Methods

Bee Collection. In spring 2019, blue orchard bee nesting materials were deployed in open landscapes to collect immature bees from two geographically distinct locations: near Logan in northern Utah (41.798, -111.650; 1,675 m), and near Leavenworth in central Washington (47.482, -120.656; 500 m) (Fig 2-1). Bundled cardboard tubes lined with paper straw inserts (diameter = 7.5-8 mm, length = 16.2 cm, end closed off by a wax or plastic plug) were housed in a corrugated plastic shelter (length by width by height = 22 × 17 × 26 cm) (as in Artz et al. 2014). These materials were used so that wild bee nests could be collected and kept intact for transport and experimentation. To increase nesting in the provided materials, a patented formulation of chemical attractant (decanoic acid dissolved in ethyl acetate) was prepared and applied to the front end of the nesting cavities (Pitts-Singer et al. 2016).

Nesting materials were checked periodically for bee activity and completion. Recently provisioned (≤2-week-old) nests were collected and shipped (WA bees) or transported (UT bees) immediately to the USDA-ARS Pollinating Insects Research Unit in Logan, Utah. There, the paper straw inserts were sliced longitudinally and visually inspected to select only brood cells with an egg, a first stadium larva inside the egg chorion, or a recently hatched second stadium larva (Table 2-1). Bees older than the second stadium were left in the nest but not further observed or used in the study. Cells were kept intact within the paper straw nests and held on corrugated cardboard trays by treatment throughout larval development (Fig. 2-2).

Treatments (bee origin and thermal regimen). For each population, the collected blue orchard bee nests were alternately assigned to one of two temperature treatments, constant or natural thermal regimen, until at least 300 individual brood cells were available for each treatment. In total, 1,432 blue orchard bee brood cells were used in the experiment, split between four treatment groups: (1) UT bees exposed to a constant thermal regimen (UT-constant, n = 373), (2) WA bees exposed to the same constant temperatures as UT bees (WA-constant, n = 369), (3) UT bees exposed to a natural thermal regimen that mimics a cherry production zone near their collection site (UT-natural, n = 325), and (4) WA bees exposed to a natural thermal regimen that mimics a cherry production zone near their collection site (WA-natural, n = 365) (Fig. 2-3).

Each treatment consisted of approximately 60 nests (UT-constant = 63, WA-constant = 60, UT-natural = 62, WA-natural = 59), and nests each contained a mean of six brood cells (mean ± SE: UT-constant = 5.9 ± 2.5, WA-constant = 6.1 ± 1.8, UT-natural = 6.2 ± 2.2, WA-natural = 6.2 ± 2.4). Additionally, cells from all positions within nests were used (nests contained anywhere from 1-11 cells) with a comparable number of cell positions being represented within each treatment and population. Therefore, all treatments contained cells that held female and male bees.

The nests in the constant thermal regimen were reared in a common environmental test chamber (Forma Scientific Dual Temperature Cabinet, Marietta, OH) set at 26°C (as in Bosch and Kemp 2000, 2001, Orchard Bee Association 2021) throughout their immature development. Thirty days after adult eclosion (mean date by bee origin), bees were cooled over a two-week period according to recommended management practices by subjecting them to 19°C for one week and then 11°C for the next week, requiring a second incubator to accommodate differences in development timing (Table 2-2) (similar to Orchard Bee Association 2021). At the end of two weeks, they were placed at a winter storage temperature of 4°C (Bosch and Kemp 2000, 2001); once the wintering temperature was reached for both populations, they were again held in a common chamber where they remained until the following spring. These management steps were made to avoid excessively long (>45 days) pre-wintering periods, which can cause high winter and pre-emergence mortality (Bosch and Kemp 2004, Bosch et al. 2008).

The natural thermal regimen reflected the use of “local” bees as pollinators in their regional orchard environments and served as a phenological comparison for each bee population to the constant thermal regimen. Nests from the UT montane collection site were placed in an incubator programmed for temperatures in a UT cherry orchard. Another incubator held WA bees and was programmed for temperatures in a WA orchard. To simulate these conditions for each bee population, ramping-temperature incubators (Percival Intellus Control System, Percival Scientific, Inc., Perry, IA, USA) were programmed to mimic the natural daily (24 h) temperature cycles of their nearest orchard growing region for which data was available, starting at average cherry bloom time (Fig. 2-1).

These programmed diel temperature cycles were updated weekly to match average hourly temperatures recorded over a recent 6-year period (2012-2017) from weather stations in Provo, Utah (40.21667, -111.71667; 1,370 m; approx. 170 km from the collection site) and Wenatchee, Washington (47.39749, -120.20121; 378 m; approx. 35 km from the collection site) (MesoWest 2021). Bees in the natural thermal regimens remained at these natural daily temperature cycles throughout immature development, winter dormancy, and adult emergence the following spring.

The timing of tart cherry bloom in central Utah and sweet cherry bloom in the Columbia Basin of Washington is similar, typically between mid to late April (USDA-NASS 2006). For the UT-natural and WA-natural treatments, the programmed diel temperature cycle for Week 1 of our experiment corresponded to mean hourly temperatures from 16 April (Day 1) to 22 April (Day 7) in each location (Table 2-2). Week 2 of the experiment corresponded to the following seven dates, and so on. Since blue orchard bees were collected from higher altitude wildlands than orchard landscapes and are naturally active in collection locations after cherry bloom, nests used in this study were made and collected after 16 April; UT bees (eggs or 2nd stadium larvae) were obtained and assigned to treatments between actual calendar dates of 14-21 May, and WA bees between actual dates of 07-14 May. For sake of ease and clarity, we have reported dates in terms of weeks and days since the start of the experiment or dates which correspond to the timing of cherry bloom (i.e., the artificial timeline).

Mortality and life stage development. Each bee cell was visually inspected (with the aid of a compound microscope, when necessary) three times per week (on Monday, Wednesday, and Friday) to document mortality and timing of immature life stages until larvae completed cocoon spinning (similar to Pitts-Singer et al. 2014) (Table 2-1). Because the date of oviposition was unknown, the first life stage date recorded for all study specimens was the second stadium larva, after eclosion from the egg chorion (Torchio 1989) – this served as a reliable starting point for comparing treatment effects on development. Once cocooned, bees were considered prepupae and transferred to individual gelatin capsules. Digital radiographic images (12 s exposure at 24 kVp; computed radiography high-resolution system by Faxitron X-Ray LLC, Linconshire, IL) were taken three times per week (Monday, Wednesday, Friday) to determine development periods for the prepupal, pupal, and adult life stages (Table 2-1).

Mortality was indicated by the failure to develop to the subsequent life stage (e.g., a bee died in the egg stage if a larva did not eclose form the egg chorion, or died in the prepupal stage if metamorphoses was incomplete). To compare mortality between treatments, we performed three Pearson's Chi-squared tests – one with respect to population origin at the constant thermal regimen (i.e., UT-constant vs WA-constant), and two more with respect to thermal regimen for each population origin (i.e., UT-constant vs UT-natural and WA-constant vs WA-natural).

To look for effects of thermal regimen and bee origin on duration of immature development (from second stadium to adult eclosion), an analysis of variance (ANOVA) was performed for each life stage by sex. The duration of the adult life stage was not included because it is largely dependent on the timing of spring emergence, which was analyzed separately; rather, we were interested in how temperature would affect the timing of management practices, which occur up until bees are put in cold storage for winter. Female and male bees were analyzed separately because life stage duration has previously been shown to vary by sex (Bosch et al. 2000, Sgolastra et al. 2012, Pitts-Singer et al. 2014). To determine sex, we visually inspected bees after emergence. Cocoons containing pupae and adults were also dissected to determine the sex as was possible. Sex could not be determined for prepupae and some pale (unsclerotized) pupae, nor for bees that died in the egg or larval stage. All analyses were run in R 4.0.0.

Emergence. The UT- and WA-constant treatments were each further subdivided into two post-winter incubation events. One subset was timed to incubate cocooned UT and WA bees in mid-March to simulate commercial management for pollinating California cherry orchards (Table 2-2); this treatment reflects a common real-world scenario in which bees are translocated because pollination demand in California is high and the natural abundance of blue orchard bees abundance is low. The other subset was timed for incubating bees on two dates in mid-April to simulate pollinating cherry orchards in their natal regions (i.e., UT bees timed for cherry bloom in UT and WA bees timed for cherry bloom in WA).

Prior to the first incubation, the test chamber containing UT-constant and WA-constant bees was raised from 4°C to 7°C for five days to prime bees for subsequent rapid emergence (Table 2-2), which is a practice used by some bee managers (cite). However, because some males emerged at 7°C (see below) the temperature was cooled to 5°C; the males that emerged prematurely were excluded from further statistical analyses. At their assigned times times, cocooned bees were moved to a 24°C incubator to induce emergence as if used for pollination. Bees were then checked daily for emergence (i.e., when the adult bee had chewed out of its cocoon but remained in the gelatin capsule). To reveal any effects of be origin on the timing of temperature-induced emergence an ANOVA was performed for each incubation event by sex.

Bees in the natural thermal regimens remained at average daily temperature cycles of their region of origin (i.e., were not manipulated via artificial conditions to induce emergence) to reveal emergence timing in the absence of temperature management. Bees were checked daily once natural temperatures reached 10°C to document date for each adult emergence. To reveal any effects of be origin on the natural emergence period an ANOVA was performed by sex.

Results

Mortality. When reared at the same constant thermal regimen, significantly more WA bees (43%) died than did UT bees (35%) ($\chi^2 = 5.29$, df = 1, p = 0.021); the increased mortality of WA bees was primarily during the larval stage (Fig. 2-4). With respect to thermal regimen, mortality was significantly higher for bees reared at natural temperatures compared to the constant temperatures, for both UT bees ($\chi^2 = 256.85$, df = 1, $p < 0.001$) and WA bees ($\chi^2 = 152.24$, df = 1, $p < 0.001$). Mortality tended to increase from early to late life stages – compared to the constant treatment, mortality was eight times higher for UT-natural prepupa and pupae and five times higher for WA-natural pupae. Due to the high mortality in the UT populations prior to reaching the adult stage, the sex was unknown for many of the Utah offspring reared but whose data was collected for determination of duration of immature life stages (see below).

Life stage development. For both bee populations and sexes, immature development (from the second stadium larva to adult eclosion) was completed in 69-78 days. When bees from both regions were reared at constant 26°C, immature development was shorter for WA bees than for UT bees; this difference was significant for females but not for males (Tables 2-3 and 2-4, Fig. 2-5). The duration of larval and prepupal stages were not statistically different by bee origin; however, the pupal stage was significantly shorter for WA bees compared to UT bees.

With respect to thermal regimen, development was shortened for both UT and WA bees when reared at the warmer constant temperature compared to the natural thermal regimen (Tables 2-3 and 2-4, Fig. 2-5). This difference was significant for WA bees but not for UT bees, of box sexes. The larval period prior to defecation was significantly reduced for bees reared at the constant compared to the natural thermal regimens, by about 13 days for UT females and 11 days for WA females; similar results were found for males. By contrast, we found that the prepupal stage was significantly prolonged, for both UT females and WA females; similar results were found for males. Overall, thermal regimen had little effect on the duration of the pupal stage for both bee origins. Similar trends in the durations of immature stages were observed from analyses of data with sex pooled, and thus included the individuals whose sex was undetermined due to mortality prior to the adult stage (Figure 2-6).

Mean rearing temperatures during immature development were higher in the constant thermal regimen (26°C) compared to the natural thermal regimen for both bee sources (Fig. 2-7). Since temperatures in the natural thermal regimens gradually increased through spring and summer, this effect of temperature was greater during the larval period than for the prepupal and prepupal period. For the UT-natural regimen, mean temperatures were 12-22°C during larval development, 19-22°C during prepupal development, and 19-26°C during pupal development. For WA-natural bees, mean rearing temperatures were cooler than UT-natural temperatures during larval development (12-18°C) and similar during prepupal and pupal development. However, maximum temperatures exceeded 26°C by the end of the larval period in the UT-natural treatment and during the prepupal period for the WA-natural treatment.

Although the exact ages of the eggs were unknown, their development to the second larval stadium was greatly affected by temperature. The time needed for eggs to hatch was responsible for a greater shift in the timing of adult eclosion than what is represented above by duration of immature development (Fig. 2-7). For both bee populations and sexes, mean adult eclosion was 9-10 days later than the duration of immature development in the constant thermal regimen and 16-30 days later in the natural thermal regimen. Unlike the constant thermal regimen in which bees were gradually cooled to wintering temperatures (4°C) 30 days after adult eclosion on Week 16) bees in the natural thermal regimen were subjected to warm temperatures for longer during the pre-wintering period. Mean temperatures did not reach the same wintering temperature until approx. 14 and 12.5 weeks later than the constant thermal regimen, for UT-natural and WA-natural treatments, respectively (Fig. 2-8).

Emergence. For the cocooned bees in the constant thermal regimen placed at 7°C (from 4°C) prior to incubation, 3♀ and 90♂ UT bees and 1♀ and 31♂ WA bees emerged prematurely and were excluded from any data analysis concerning adult emergence timing (Table 2-2). When one subsample of bees was managed (incubated at 24°C) for March orchard bloom (UT bees on Day 339 and WA bees on Day 333), over 90% of UT and WA bees to emerge did so within the first and second day after incubation (Fig. 2-8, Table 2-7). When the other subsample of bees was incubated in April (UT bees on Day 365 and WA bees on Day 360) to mimic the same timing of orchard bloom in their respective regions, 88 and 98% of UT and WA bees, respectively, emerged within 1-2 days.

When UT and WA bees were reared at natural orchard temperatures of their respective regions, they emerged over a much longer period than bees in the constant thermal regimen that were induced (Fig. 2-8, Table 2-7). Bees in the UT-natural group emerged over a 32-day period, 85% of which emerged between Day 350 (peak male emergence) and Day 366 (peak female emergence). Bees in the WA-natural group emerged over a 26-day period, 95% of which emerged between Day 344 (peak male emergence) and Day 360 (peak female emergence).

Discussion

This study revealed the effects of geographic origin and rearing temperature on blue orchard bee development and survival. Use of the common, constant thermal regimen for UT- and WA-sourced bees allowed for isolating the differences between the populations in the absence of temperature variation. Use of the natural orchard temperatures allowed a comparison of development and survival for each population at both the constant and natural temperatures to reveal impacts of realistic uses of bees as pollinators in their respective localities.

Temperatures are expected to affect developmental rates of ectothermic organisms. The general effect is that a rise in temperature would hasten development, but that an extreme increase in temperature would be harmful. However, responses to rearing temperature can vary by species and populations within species (Colinet et al. 2015, Rebaudo and Rabhi 2018, Forrest et al. 2019, Orr et al. 2021). This work corroborates these two former statements. At the constant warm temperature, young UT and WA larvae fed and grew very quickly. The orchard temperatures of both states during larval development times fluctuated but were much lower than the constant 26°C, meaning that larval growth was hastened under the artificially warm constant conditions. This trend did not hold for the prepupal and pupal stages, in which mean orchard temperatures were still cooler than the constant 26°C, but maximum temperatures exceeded 30°C, and the prepupal period was prolonged and there was little effect on the pupal stage.

Despite a reduction in immature development time, overall bee mortality was low under constant thermal regimen. The level of larval mortality in this study (<20%) was similar to a previous laboratory study with UT bees held at 22 and 26°C (Bosch and Kemp 2000). However, there were differences between these two bee populations in survival and developmental phenology when reared at the same temperatures. Interestingly, mortality for WA bees was higher than for UT bees at the larval stage. The WA bees also were quicker to develop to adulthood due to shorter prepupal and pupal stages, especially for the female bees. Together, these results suggest that WA bees may be more adversely affected by the warmer than natural spring and early summer conditions than UT bees.

At natural orchard temperatures, mortality was higher for both bee populations than when reared at constant temperatures. The greatest mortality was in the life stages that experienced the highest maximum temperatures, which were the prepupal and pupal stages for UT bees and the pupal and adult stages for the WA bees. Bosch and Kemp (2000) found that some bees from northern UT failed to complete prepupal dormancy only at the lowest temperatures tested (constant 18°C). However, our UT-natural bees experienced a range of temperatures that were well above and just below 18°C. Although there was a decline in UT temperatures during the prepupal stage, it was only about as cool as the hottest temperature during the WA prepupal stage. McCabe et al. (2022) also found that maximum temperatures were likely to predict prepupal and pupal mortality in megachilids in natural montane environments.

Differences by bee origin in timing of development also resemble the results found by Pitts-Singer et al. (2014). In this study and the previous one, WA bees took slightly longer to reach adulthood than UT bees when reared at the same temperatures; the duration of the UT prepupal period was much shorter and pupal period longer than the durations of those life stages for WA bees. Bosch et al. (2010) found that when bees remained at warm temperatures in their adult stage for greater than 30 days, their fat bodies were depleted, and adults were less likely to emerge. If bees from different origins are managed together, this quicker development for WA bees could lead to lethal or sublethal effects if care is not taken to separately manage bee populations. Thus, farmers and bee managers could be releasing suboptimal pollinators in their orchards.

Managing bees via artificial conditions to time their release for commercial pollination is thought to be more effective than allowing for natural emergence (Bosch and Blas 1994, Bosch et al. 2000, 2008, Bosch and Kemp 2000). Similarly in this study, the use of a constant thermal regimen for both an early and late cherry bloom event resulted in synchronous and quick bee emergence from cocoons. The natural emergence of bees from both UT and WA was prolonged but occurred close to one year since they were collected as eggs, which was around the time of natural orchard bloom. Males in the natural thermal regimen emerged slightly ahead of cherry bloom, and females emerged more or less in synchrony with the timing of cherry bloom. Naturally emerging bees would benefit from supplemental, early blooming floral resources to sustain early emerging male bees prior to female emergence. After crop bloom has ceased, females could continue nesting if floral resources are available (Boyle et al. 2020).

When used for orchard pollination, bee nests typically remain within the crop during much of the egg and larval stage. In this study, there was relatively low mortality for both UT and WA bees at both thermal regimens at these early life stages. Overall, these results have positive management implications in that use of artificial conditions are not necessary for at least 7-9 weeks after nesting. Removing nests from the field and placing them at cooler than ambient extreme temperatures once nesting has ceased should be encouraged to reduce mortality at the prepupal, pupal, and adult stages and to avoid incidence of pests or exposure to pesticides. This strategy would work well as bee nesting materials could be removed from orchards to get them out of the way of orchard maintenance and harvest activities. Maintaining bees are at a constant temperature, such as 26°C prior to reaching adulthood, might also better synchronize bees reaching adulthood so that the timing for winter storage is easy to predict and accommodate, especially when the target, pollinator-dependent crop blooms in early spring.

The detrimental effect of the higher-than-average temperatures late summer implies that using bees in climates with temperatures warmer than, or at the extreme ends of, their native temperature ranges can be detrimental for developing bees. Scenarios where temperature extremes are experienced by blue orchard bees are realistic, as in California for almond pollination when bees are left to develop there. If bees from Utah and Washington produce offspring in California during February almond bloom, then many of those progenies are unlikely to survive if not managed for much of the year under controlled temperatures. Thus, study suggests that bees from the same geographic region as the crop in which they are being utilized for pollination services may also require management at artificial conditions for optimizing survival and performance.

Understanding how temperature affects solitary bees managed to provide pollination services can help to not only assure population survival, but also inform how to use temperature management for timing adult bee emergence with crop bloom, especially when the crop is located where climate differs from that of the bees' geographic origin (Bosch et al. 2000, Sgolastra et al. 2011, Pitts-Singer et al. 2014). Further research is needed to understand whether geographically distinct bee populations present different physiological thermal tolerances, differences in developmental timing of specific life stages, or differences in ability to adapt to novel environments over generations, when extreme climatic conditions are experienced, such as prolonged or shortened summer quiescence as prepupae and earlier or later spring adult emergence.

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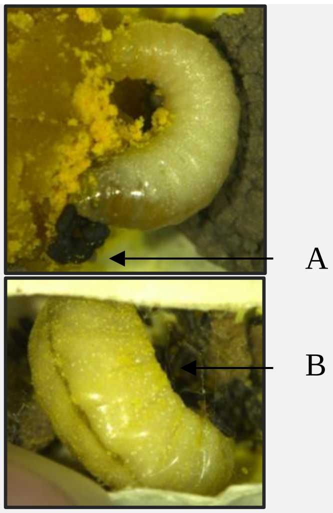
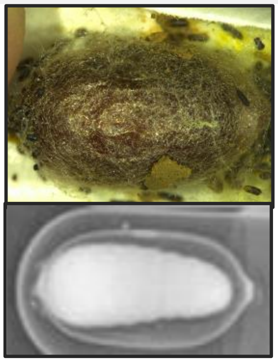
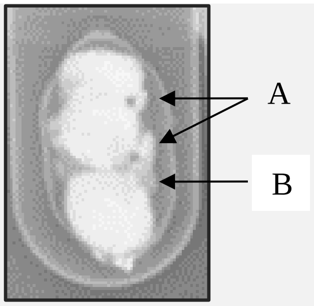
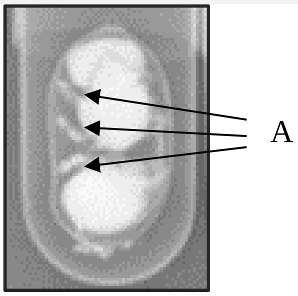
Tables

Table 2-1

Life Stages Used to Document Development Timing

Life Stage	Experiment Details	Diagnostics	Figure(s)
Egg	Brood cells with only eggs or recently (<48h) hatched larvae were selected.	The developing embryo and subsequent first larval stadium is contained within the egg chorion (Torchio 1989).	
Second Stadium Larva	Hatched larvae with only little or no pollen in the gut were selected	Once eclosed, second stadia are nearly translucent, having little to no pollen in the gut. Larvae begin to feed within 24h of hatching (Torchio 1989).	
Fifth Stadium Larva	The timing of first fecal pellets and silk were record.	The first fecal pellets (A) are deposited within 24h of molting to the fifth stadium (Torchio 1989). Cocoon building is initiated <24h after the pollen provision is consumed (Helm et al. 2017). Larvae make circular motions to weave fine, white silk strands (B) produced from glands near the mouth, around its body. Cocoons darken and harden with each layer.	
Prepupa	Completed cocoons marked the start of the prepupal stage.	Cocoon building is complete when the exterior is dark and rigid. Prepupa (cocooned fifth stadium larvae) undergo a month-long dormancy (Bosch and Kemp 2001). By radiographic imaging, prepupae first appear C-shaped, then straighten before undergoing metamorphosis.	

Pupa	Radiographic images revealed the timing of metamorphosis.	Three-segmented pupae are easily distinguished from the grub-like prepupal form after metamorphosis. The legs (A) of pupae are held close to the body and developing wings (B) appear opaque laterally and posteriorly from the thorax.	
Cocooned adult	Radiographic images were used to determine the timing of adult eclosion.	After adult eclosion, fully sclerotized legs (A) may splay laterally, and developed wings are translucent. The thorax and head capsule are well-defined.	
Emerged adult	When bees were fully egressed from the cocoon.	Bees initiate emergence by chewing a hole in the cocoon, using their mandibles, to crawl out.	

Note. Blue orchard bees originating from Utah and Washington were reared within intact nests during immature development, and then transferred to gel capsules once cocoons were completed to continue tracking development via radiographic imaging. The following life stages were used to track development throughout their entire life cycle.

Table 2-2

Timeline of Experimental Events

Event	Artificial Timeline	Day of Experiment
Intake first batch of WA (n=233) and UT bee cells (n=426). Nests were assigned to constant or natural thermal regimen.	16 April, Year 1	Day 1
Intake second batch of WA (n=501) and UT bee cells (n=272). Bees assigned to treatments similar to above.	23 April, Year 1	Day 8
Mean day of adult eclosion for the WA-constant group.	04 July, Year 1	Day 80
Mean day of adult eclosion for the UT-constant group.	06 July, Year 1	Day 82
Wintering process initiated for the WA-constant group by lowering rearing temperature from 26°C to 19°C.	03 August, Year 1	Day 110
Wintering process initiated for the UT-constant group by lowering rearing temperature from 26°C to 19°C.	06 August, Year 1	Day 113
WA-constant rearing temp. lowered from 19°C to 11°C.	13 August, Year 1	Day 120
UT-constant rearing temp. lowered from 19°C to 11°C.	16 August, Year 1	Day 123
WA-constant rearing temp. lowered to 4°C for winter.	23 August, Year 1	Day 130
UT-constant rearing temp. lowered to 4°C for winter.	26, August, Year 1	Day 133
Wintering temp. raised from 4°C to 7°C to prepare for subsequent incubation of UT-constant group.	04 March, Year 2	Day 324
Temp. for bees in the UT-constant group lowered to 5°C, as bees (2♀ and 91♂) prematurely emerged at 7°C.	09 March, Year 2	Day 329
Wintering temp. raised from 4°C to 7°C to prepare for subsequent incubation of WA-constant group.	11 March, Year 2	Day 331
Subset of WA-constant group incubated at 24°C to induce emergence as if used for pollination in CA cherry orchard.	13 March, Year 2	Day 333
Temp. for remaining WA-constant bees lowered to 5°C, as bees (1♀ and 31♂) prematurely emerged at 7°C.	16 March, Year 2	Day 336
Subset of UT-constant group incubated at 24°C to induce emergence as if used for pollination in CA cherry orchard.	19 March, Year 2	Day 339
Subset of WA-constant group incubated at 24°C to induce emergence as if used for pollination in WA cherry orchard.	09 April, Year 2	Day 360
Subset of UT-constant group incubated at 24°C to induce emergence as if used for pollination in UT cherry orchard.	14 April, Year 2	Day 365

Table 2-3

Mean, Standard Error, and Sample Size for Development Duration of Immature Life Stages by Treatment

Life Stage	UT-Constant	WA-Constant	UT-Natural	WA-Natural
		Male Bees		
Immature (2nd Stadium to Adult)	70.46 ± 0.45 n = 145	68.64 ± 0.75 n = 121	72.00 ± 1.08 n = 9	73.56 ± 0.45 n = 97
Larva (2nd Stadium to Prepupa)	22.80 ± 0.25 n = 147	23.33 ± 0.59 n = 126	33.00 ± 0.42 n = 10	33.01 ± 0.34 n = 110
2nd to 5th Stadium	3.89 ± 0.10 n = 147	3.35 ± 0.12 n = 126	16.80 ± 0.33 n = 10	14.11 ± 0.22 n = 110
5th Stadium to Cocoon	11.16 ± 0.25 n = 147	9.54 ± 0.29 n = 126	12.30 ± 0.50 n = 10	12.79 ± 0.22 n = 110
Cocoon to Prepupa (Cocoon Building)	7.74 ± 0.32 n = 147	10.00 ± 0.66 n = 126	4.00 ± 0.38 n = 10	6.00 ± 0.20 n = 110
Prepupa (Prepupa to Pupa)	18.61 ± 0.39 n = 147	17.34 ± 0.53 n = 126	10.90 ± 0.50 n = 10	14.64 ± 0.30 n = 110
Pupa (Pupa to Adult)	29.03 ± 0.22 n = 145	28.05 ± 0.26 n = 121	28.44 ± 0.91 n = 9	26.51 ± 0.27 n = 97
		Female Bees		
Immature (2nd Stadium to Adult)	74.50 ± 0.55 n = 117	70.14 ± 0.60 n = 118	77.08 ± 1.16 n = 12	77.69 ± 0.54 n = 101
Larva (2nd Stadium to Prepupa)	24.33 ± 0.25 n = 121	24.21 ± 0.55 n = 120	35.42 ± 0.81 n = 12	35.47 ± 0.34 n = 155
2nd to 5th Stadium	3.76 ± 0.10 n = 121	3.70 ± 0.13 n = 120	16.50 ± 0.62 n = 12	15.01 ± 0.19 n = 155
5th Stadium to Cocoon	13.62 ± 0.29 n = 121	9.63 ± 0.31 n = 120	14.25 ± 0.59 n = 12	13.82 ± 0.16 n = 155
Cocoon to Prepupa (Cocoon Spinning)	6.95 ± 0.35 n = 121	11.00 ± 0.66 n = 120	5.00 ± 0.48 n = 12	7.00 ± 0.21 n = 155
Prepupa (Prepupa to Pupa)	18.08 ± 0.43 n = 121	16.94 ± 0.50 n = 120	9.25 ± 0.95 n = 12	14.11 ± 0.27 n = 154
Pupa (Pupa to Adult)	32.19 ± 0.32 n = 117	28.82 ± 0.26 n = 118	32.42 ± 1.22 n = 12	28.41 ± 0.39 n = 101

Table 2-4

Results From Analyses of Variance for Development Duration of Immature Life Stages by Treatment

Life Stage	**Constant UT : WA**	**Natural UT : WA**	**UT bees Con : Nat**	**WA bees Con : Nat**
Male Bees				
Immature (2nd Stadium to Adult)	P = 0.09 Δ = 1.82	P = 0.89 Δ = -1.56	P = 0.89 Δ = -1.54	*P = 0.00 Δ = -4.91
Larva (2nd Stadium to Prepupa)	P = 0.78 Δ = -0.53	P = 0.99 Δ = -0.01	*P = 0.00 Δ = -10.20	*P = 0.00 Δ = -9.68
2nd to 5th Stadium	*P = 0.03 Δ = 0.54	*P = 0.00 Δ = 2.68	*P = 0.00 Δ = -12.91	*P = 0.00 Δ = -10.77
5th Stadium to Cocoon	*P = 0.00 Δ = 1.62	P = 0.96 Δ = -0.49	P = 0.62 Δ = -1.14	*P = 0.00 Δ = -3.25
Cocoon to Prepupa (Cocoon Building)	*P = 0.00 Δ = -2.70	P = 0.53 Δ = -2.20	*P = 0.08 Δ = 3.84	*P = 0.00 Δ = 4.34
Prepupa (Prepupa to Pupa)	P = 0.13 Δ = 1.26	*P = 0.08 Δ = -3.74	*P = 0.00 Δ = 7.71	*P = 0.00 Δ = 2.70
Pupa (Pupa to Adult)	*P = 0.02 Δ = 0.59	P = 0.17 Δ = 1.94	P = 0.92 Δ = 0.59	*P = 0.00 Δ = 1.54
Female Bees				
Immature (2nd Stadium to Adult)	*P = 0.00 Δ = 4.42	P = 0.99 Δ = -0.61	P = 0.50 Δ = -2.52	*P = 0.00 Δ = -7.56
Larva (2nd Stadium to Prepupa)	P = 0.99 Δ = 0.13	P = 0.99 Δ = 0.05	*P = 0.00 Δ = -11.08	*P = 0.00 Δ = -11.26
2nd to 5th Stadium	P = 0.99 Δ = 0.05	*P = 0.03 Δ = 1.49	*P = 0.00 Δ = -12.75	*P = 0.00 Δ = -11.31
5th Stadium to Cocoon	*P = 0.00 Δ = 4.04	P = 0.95 Δ = 0.43	P = 0.90 Δ = -0.59	*P = 0.00 Δ = -4.19
Cocoon to Prepupa (Cocoon Building)	*P = 0.00 Δ = -3.95	P = 0.51 Δ = -1.97	P = 0.38 Δ = 2.28	*P = 0.00 Δ = 4.26
Prepupa Prepupa to Pupa)	P = 0.22 Δ = 1.16	*P = 0.00 Δ = -4.86	*P = 0.00 Δ = 8.85	*P = 0.00 Δ = 2.83
Pupa (Pupa to Adult)	*P = 0.00 Δ = 3.34	*P = 0.00 Δ = 4.01	P = 0.99 Δ = -0.25	P = 0.81 Δ = 0.41

Note. Duration is in days, p-values are adjusted, and significant differences are marked with an asterisk.

Table 2-5

Summary Data and Results from Analyses of Variance for Emergence Timing and Duration by Treatment

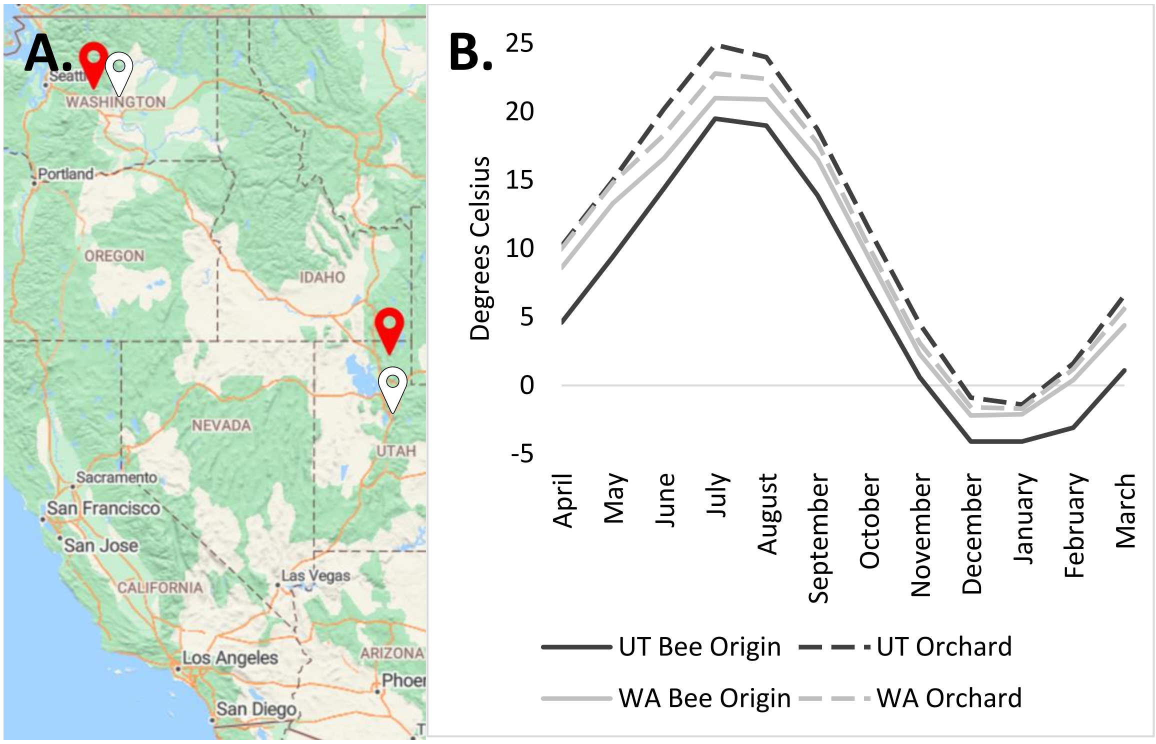
Thermal Regimen	Bee Source (No. of bees)	Adult Age	Emergence Duration (d)	Statistics (d.f. =1)
Male Bees				
Constant – March Bloom	UT (19) WA (55)	258 253	4 3	F = 7.72 p = 0.006
Constant – April Bloom	UT (24) WA (18)	284 284	7 3	F = 3.28 p = 0.071
Natural	UT (9) WA (27)	249 251	20 14	F = 1.037 p = 0.316
Female Bees				
Constant – March Bloom	UT (51) WA (50)	256 255	6 3	F = 1.30 p = 0.257
Constant – April Bloom	UT (44) WA (52)	281 280	7 3	F = 45.67 p = 0
Natural	UT (12) WA (21)	263 263	12 23	F = 0 p = 0.989

Note. Statistics were performed on the age of cocooned adults (i.e., number of days since adult eclosion in late summer) to emergence in spring. P-values are adjusted.

Figures

Figure 2-1

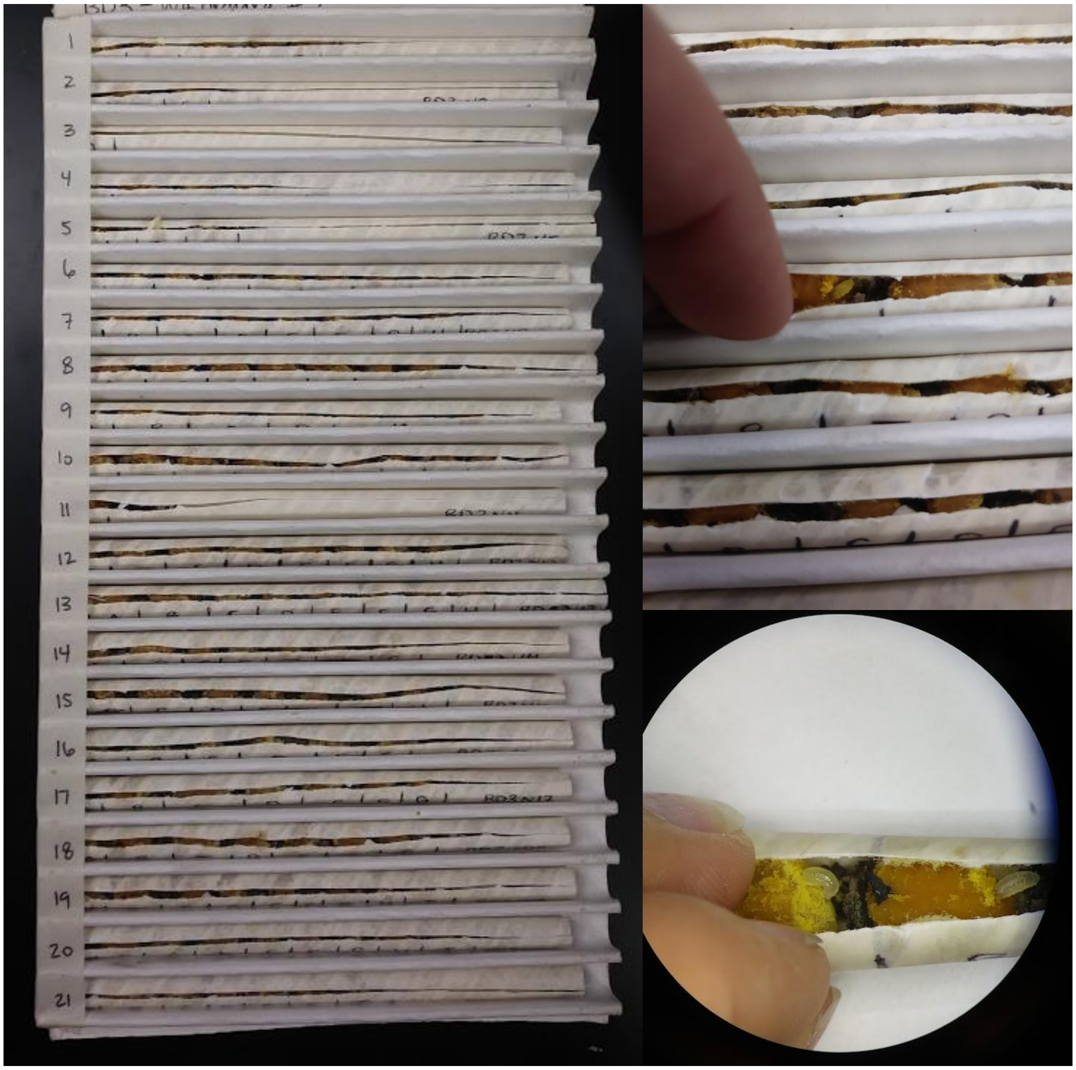
Locations and Climate Data for Bee Collection Sites and Cherry Orchard Growing Regions



Note. For a study on blue orchard bee development, (A) red drop pins mark the bee collection locations, and white drop pins mark the locations of weather stations that were used in this experiment to simulate outdoor temperatures of a cherry-growing region, and (B) mean historic monthly temperatures (from the period 1991-2020, PRISM Climate Group) of the natal location (solid line) and mean monthly temperatures (averaged over a recent 6-year period, 2012-2017; MesoWest 2021) of a cherry orchard near their source location (dashed line).

Figure 2-2

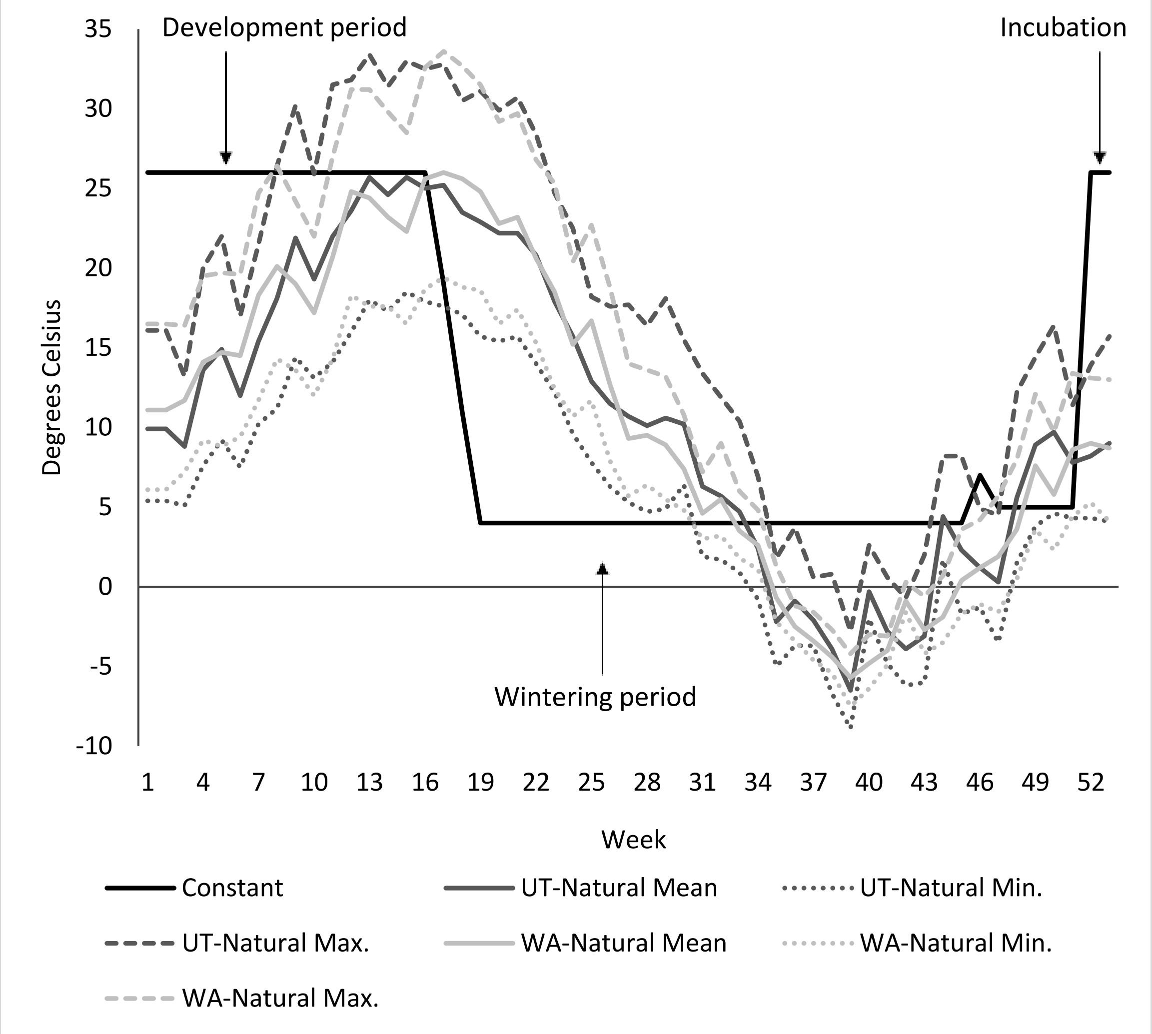
Nests and Materials Used to Track Life Stage Development



Note. A corrugated cardboard tray (left) was used to hold the paper straw nests containing blue orchard bee brood cells – individual units containing a provision of pollen and nectar and an immature bee delineated by mud partitions. Paper straws were sliced for observing bee life stages via visual inspection with the naked eye (top right) or with the use of a compound microscope (bottom right).

Figure 2-3

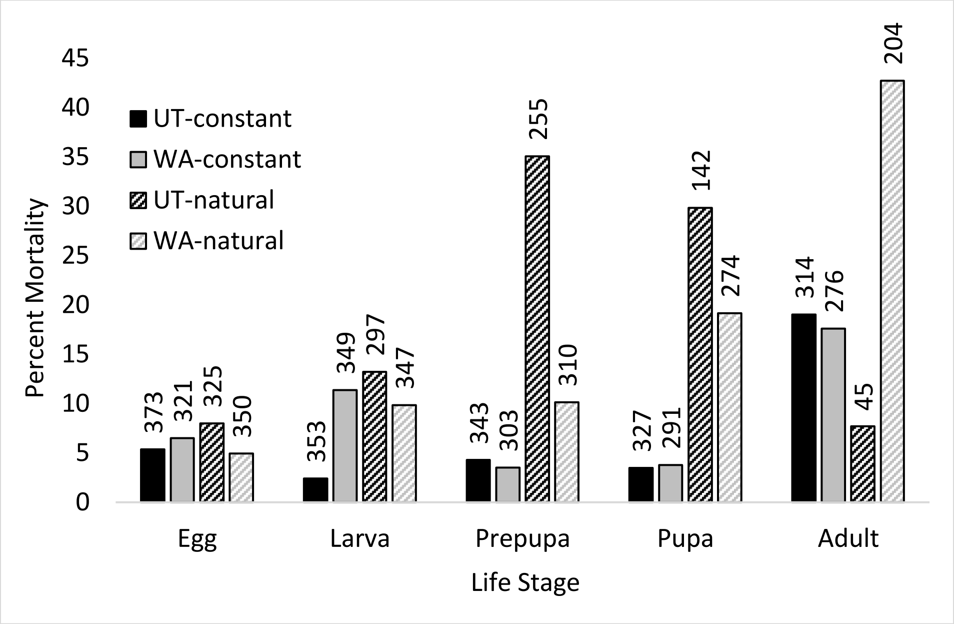
Rearing Temperatures by Treatment Group



Note. Programmed weekly temperatures for study of blue orchard bee survival, development, and emergence. Utah and Washington bees were both reared at 1) the constant thermal regimen, and 2) the mean annual temperatures from a cherry orchard near their collection site. The constant temperature was programmed to standard rearing temperatures; immature development were warm, lowered 30 days after adult eclosion to provide a moderate pre-wintering period and a long overwintering period, and temperature was increased to induce adult emergence in spring. Bees in the natural thermal regimen (UT-natural and WA-natural) were reared at diel temperature cycles (hourly temperatures averaged over a recent 6-year period, 2012-2017; MesoWest 2021) throughout their entire life cycle.

Figure 2-4

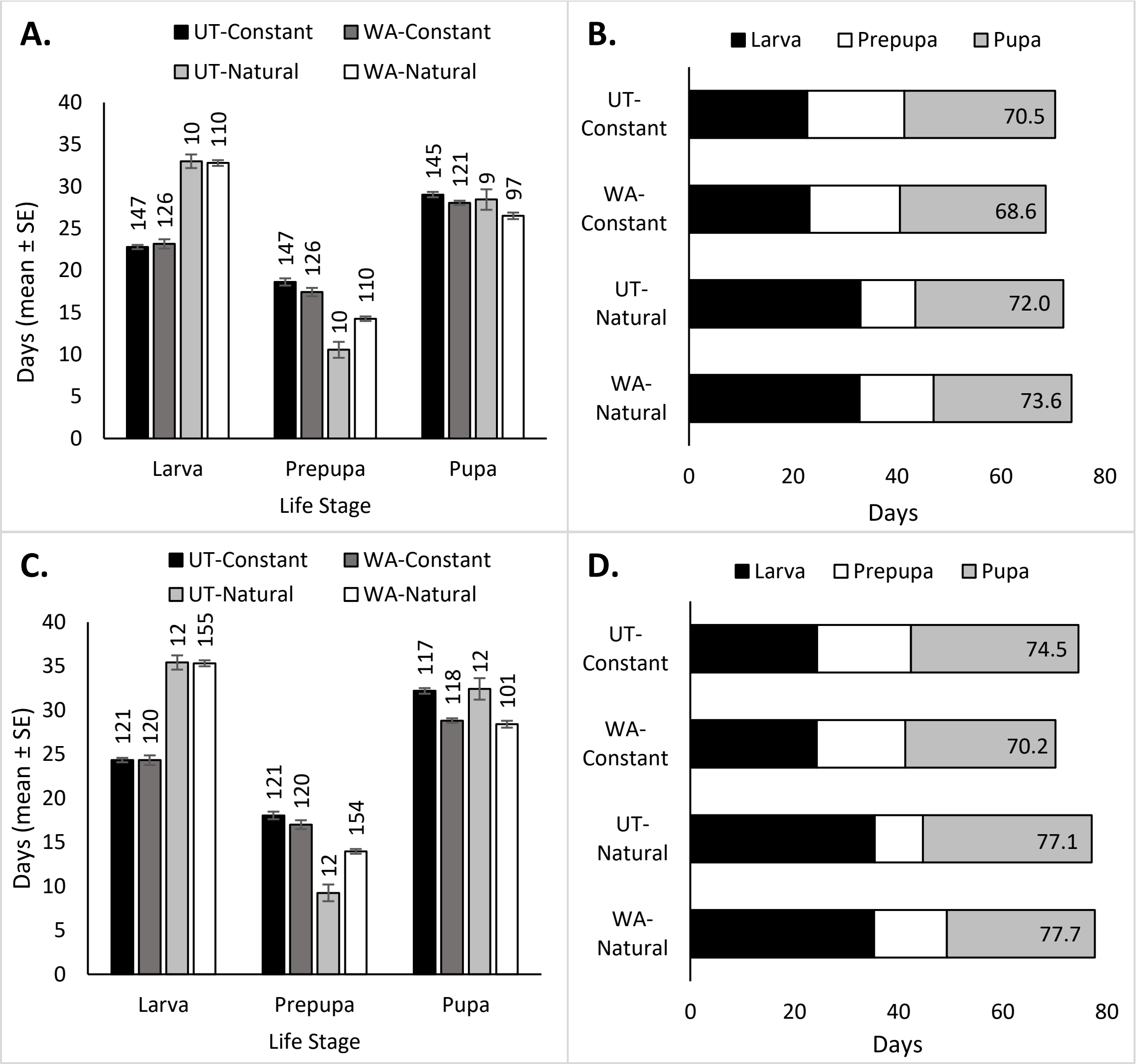
Bee Mortality by Treatment



Note. For Utah- and Washington-sourced blue orchard bees in a laboratory rearing experiment, the percent that died at each immature (egg, larva, prepupa, pupa) and mature (cocooned adult) life stage by treatment (bee origin and thermal regimen). The number of bees in each sample is noted at the top of each bar.

Figure 2-5

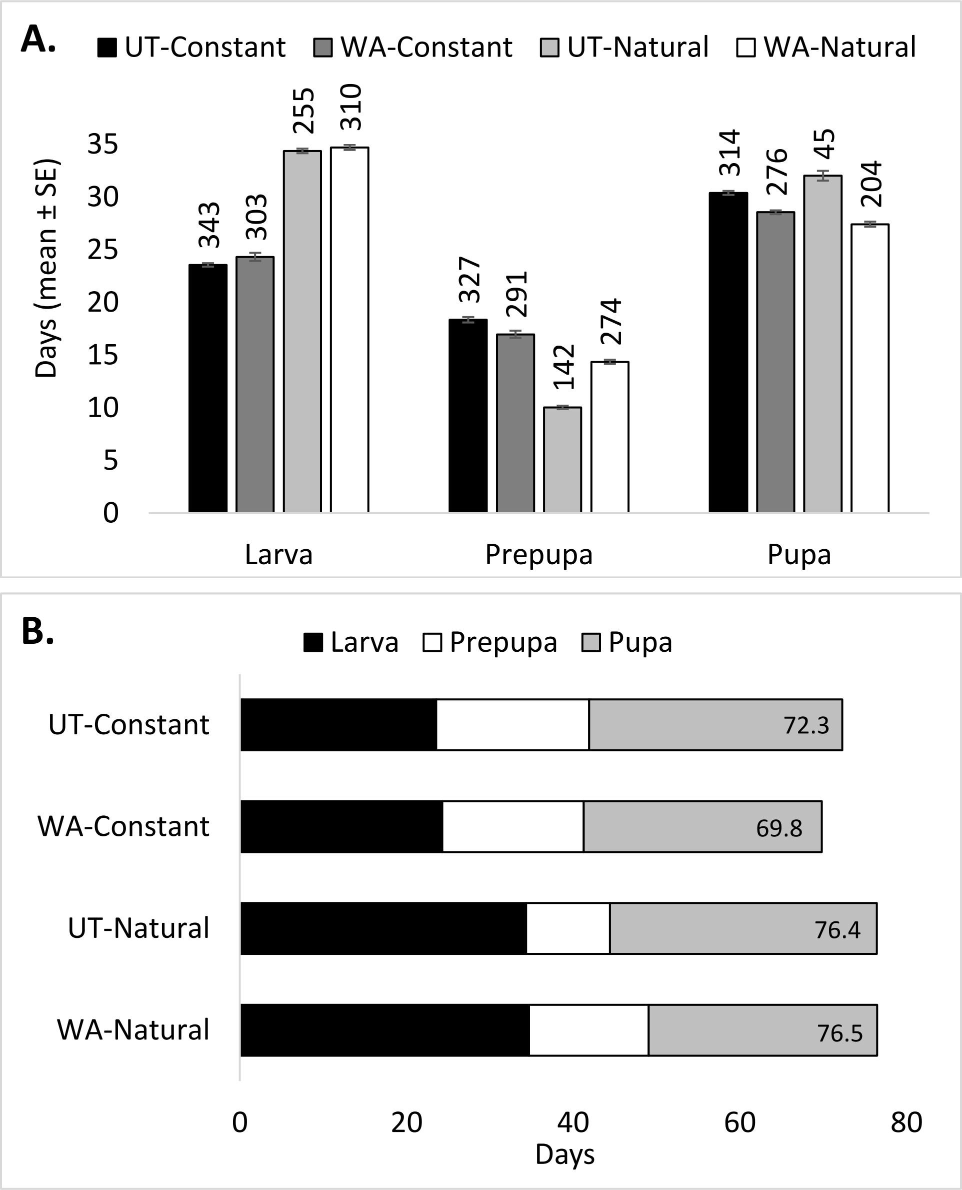
Mean Immature Life Stage Duration by Sex and Treatment



Note. For male (A, B) and female (C, B) blue orchard bees by treatment group (bee origin and thermal regimen), the mean number of days spent in each immature life stage observed. At the end of each bar, the number of bees in each sample (A, C) and the mean number of development days from the second larval instar to adult eclosion (B, D).

Figure 2-6

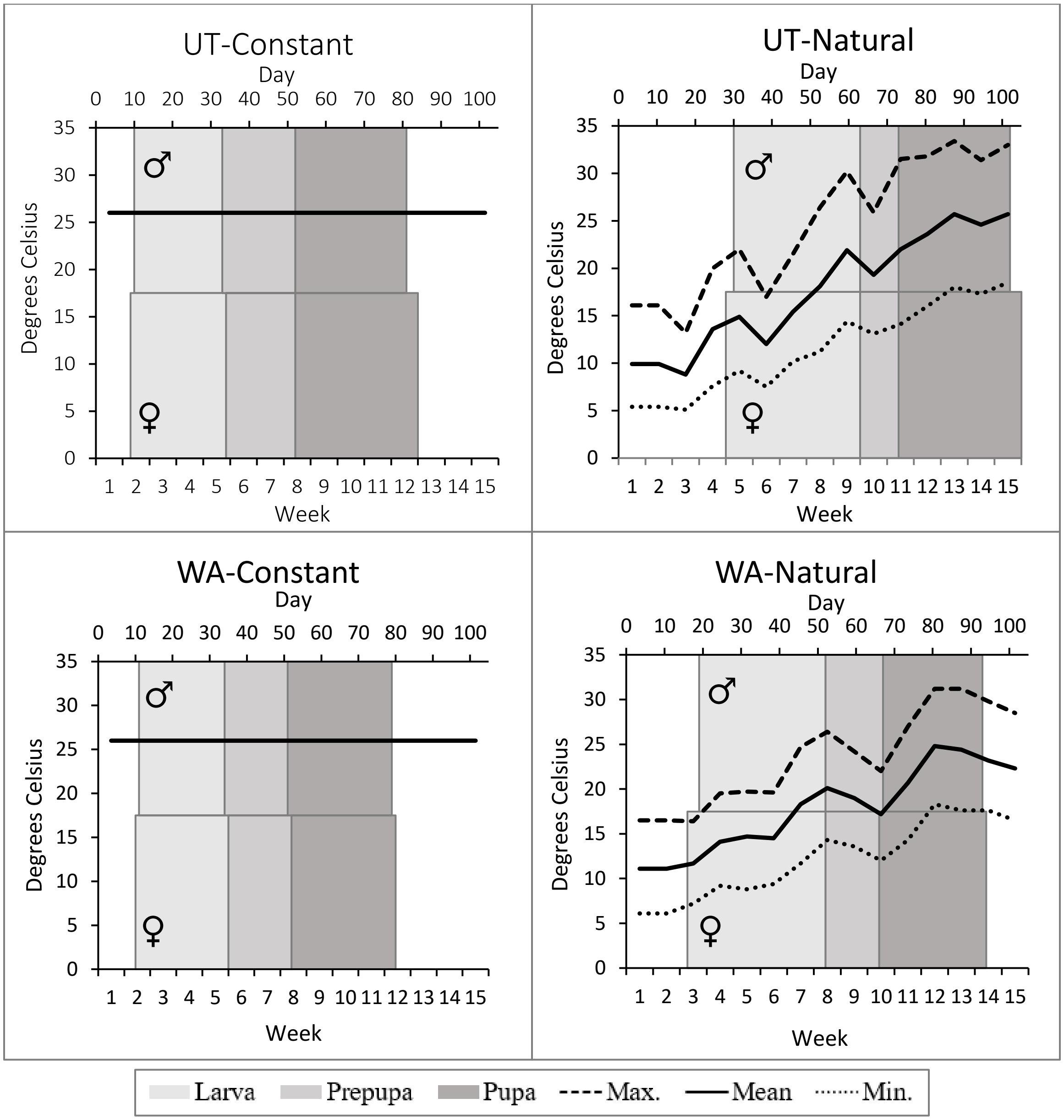
Mean Life Stage Duration by Treatment



Note. For blue orchard bees by treatment (bee origin and thermal regimen) with sex pooled, is unknown by treatment group, the number of days spent in each developmental life stage, the mean number of days spent in each immature life stage observed. At the end of each bar, (A) the number of bees in each sample and (B) the mean number of development days from the second larval instar to adult eclosion.

Figure 2-7

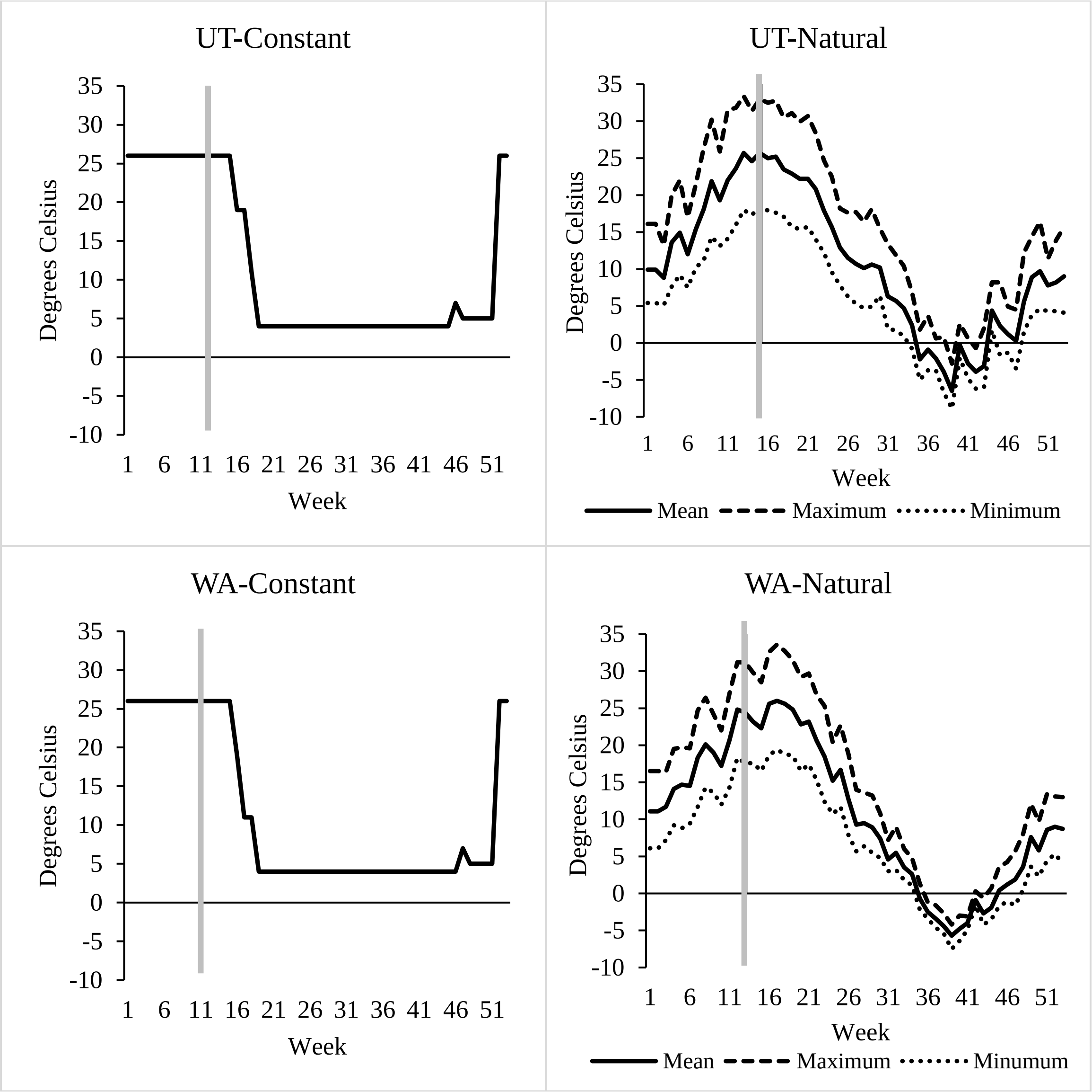
Relationship of Bee Development and Rearing Temperature



Note. By treatment group (bee source and thermal regimen), the programmed temperatures and mean duration of blue orchard bee developmental life stages.

Figure 2-8

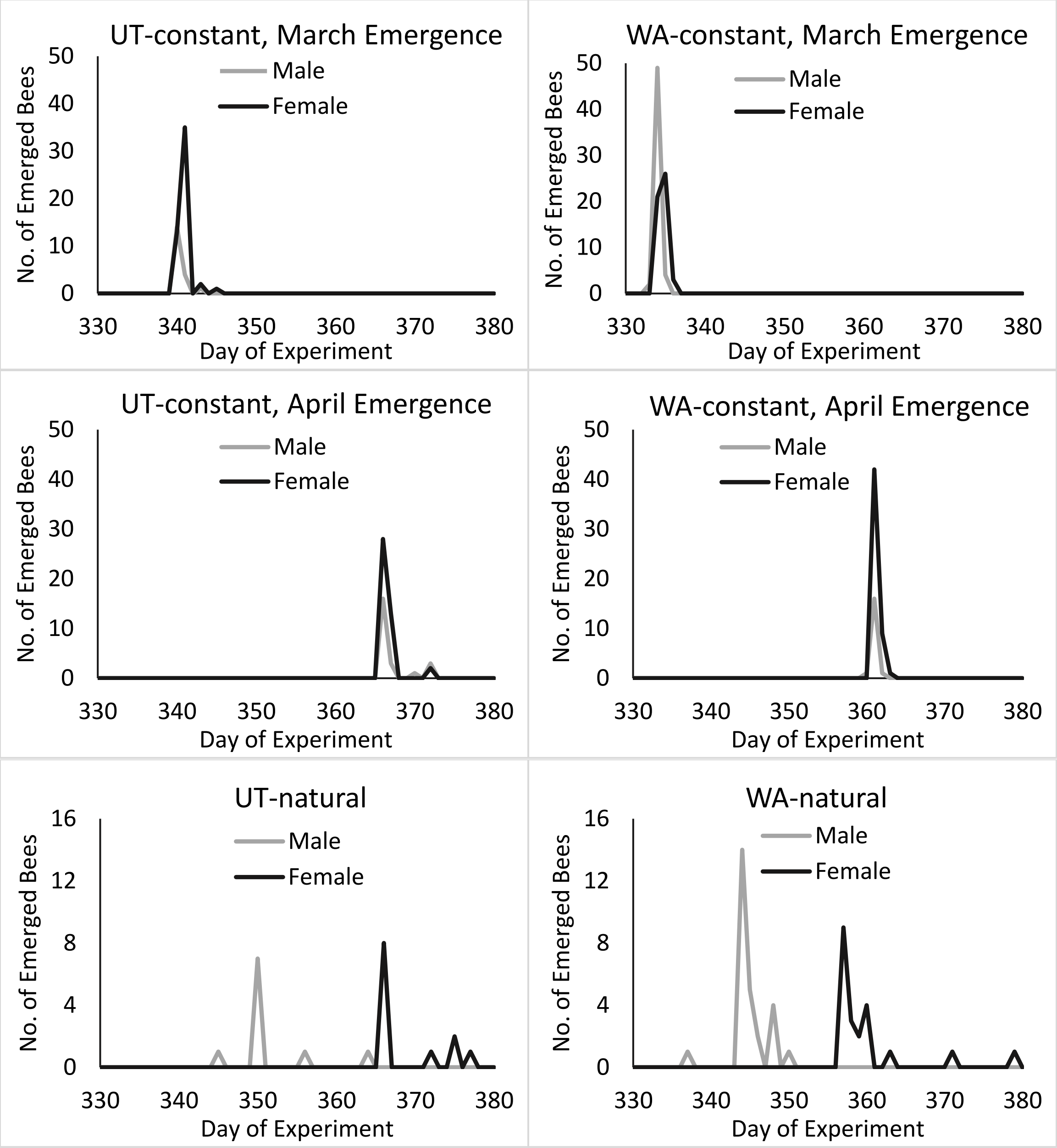
Relationship of Adult Eclosion and Thermal Regimen



Note. By treatment (bee origin and thermal regimen), the programmed weekly temperatures of environmental chambers containing blue orchard bees to track their immature development and duration of adult life stage until natural or incubation-induced emergence from cocoons. The vertical line indicates the timing of adult eclosion (with sex pooled).

Figure 2-9

Timing of Emergence by Treatment



Note. For the constant thermal regimen, bees were incubated at 24°C to induce emergence in (a, B) mid-March (UT bees on Day 339 and WA bees on Day 333), and (C, D) mid-April (UT bees on Day 365 and WA bees on Day 360). Bees in the natural thermal regimens were not induced to emerge but remained at simulated outdoor orchard temperatures (E, F); if kept for pollination in their natal orchard locations, target emergence period would be around Day 365 given similar timing of crop bloom. Prior to incubation, 3 female and 90 male UT bees emerged prematurely (at 7°C), and 1 female and 31 male WA bees emerged prematurely and are not included here.

CHAPTER III

MIXING GEOGRAPHICALLY DISTINCT BLUE ORCHARD BEE POPULATIONS TO COMPARE NESTING, REPRODUCTIVE SUCCESSS, AND EMERGENCE

Abstract

The blue orchard bee (*Osmia lignaria*) is a solitary, cavity-nesting bee that is being managed for commercial pollination of rosaceous crops in the United States. Regional differences in developmental phenology have recently been documented in populations from warmer climates such as in central California compared to colder climates such as in northern Utah. However, little is known about the effects of mixing geographically distinct populations in ways that impact their pollinator performance, such as reproductive success and synchrony of emergence with crop bloom. In March 2018, female and male bees from the same (CA♀ × CA♂, UT♀ × UT♂) and reciprocal (CA♀ × UT♂, UT♀ × CA♂) populations were released in screen cages in a plot of phacelia in central California. Uniquely paint-marked females were observed to determine nesting activity and progeny production. In 2019, surviving F1 progeny from the four crosses were flown in cages as in 2018, and the same data collected along with adult emergence. In 2018, we found that blue orchard bees sourced from UT were twice as likely to establish nests within our screened field cages. However, of the bees that did nest, we found no statistically significant differences in reproductive potential, time to initiate nest building, and the duration of nesting. Winter mortality was high due to pests, but the progeny of all four cross types maintained the natural temporal pattern of emergence by sex (males before females) the following spring. In 2019, we found no observable trends in the same metrics of pollinator performance by cross types. For bee managers and

orchardists who employ blue orchard bees from various sources, these results help alleviate concerns over mating, nesting, and reproductive success. Other concerns need to be addressed such as between year performance of bee populations trapped annually from the same location and offspring loss due to parasitoids.

Introduction

The availability of diverse wild and managed bees is essential for providing nutrients to human populations. The historic reliance on the European honey bee (*Apis mellifera* Linnaeus [Hymenoptera: Apidae]) as the sole managed bee of most pollinator-dependent crops is risky from both economic and environmental perspectives. In addition to providing habitat for wild bees, one strategy to assure profitable yields of pollinator-dependent crops is to employ other bee species that are also being commercially managed for pollination services (Isaacs et al. 2017). For food security, additional (non-*Apis* spp.) pollinators should be employed to attain economically viable and environmentally sustainable crop production.

The blue orchard bee (*Osmia lignaria* Say [Hymenoptera: Megachilidae]) has recently become available as a commercially managed pollinator of spring-blooming crops, a particularly high-value and pollinator-dependent sector of agricultural production. It is an excellent North American native pollinator of food crops such as almond, apple, cherry, plum, and pear (Torchio 1985, 2003, Brittain et al. 2013, Artz et al. 2013, Pitts-Singer et al. 2018). Blue orchard bees readily nest in artificial materials, prefer to visit rosaceous plants, and are naturally active in the spring, allowing them to be managed for and propagated in commercial orchards (Bosch and Kemp 2001). Field trials have repeatedly demonstrated their pollinating efficacy and a positive return on bee population (Torchio 1981, 1985, Bosch and Kemp 1999, 2001, Bosch et al. 2006, Boyle and Pitts-Singer 2017, 2019, Pitts-Singer et al. 2018).

Commercial management for this solitary, cavity-nesting pollinator is similar to the pollination system used for the intensively managed alfalfa leafcutting bee (*Megachile rotundata* Fabricius [Hymenoptera: Megachilidae]) (Pitts-Singer and Cane 2011). The grower typically purchases the bees and nesting materials, and manages them on their farm for pollination. Blue orchard bees are available for purchase from commercial mason beekeepers, who may also provide consultation on how to use and manage this solitary pollinator. Due to the gregarious and univoltine life history of the blue orchard bee, their management requires far less input than what is needed to maintain honey bee colonies year-round.

These inputs include nesting materials, an environment with abundant floral resources and moist soil for bee reproduction during the nesting season, and a facility for storage (climate-controlled if bees are not reliably managed at ambient temperatures). The initial population is also an investment, and optimizing reproduction in orchards reduces the cost of purchasing bees each year (given a population deficit). Reproduction can be optimized by rearing bees according to established protocols that allow for prompt and synchronous emergence with crop bloom and ensuring that supplemental forage is available after crop bloom has ceased (Bosch et al. 2008, Boyle et al. 2020). These efforts are needed for sustaining managed populations and reducing the need to trap bees from wildlands.

Because the blue orchard bee industry is relatively new, the supply is limited. Most blue orchard bee stocks are sourced from wildlands in Utah, Idaho, Washington, and Oregon, and the primary market is for use in almond production in California. Regional differences in developmental phenology have recently been documented such that the timing of management practices would need to be adjusted for optimizing survival. For example, California bees have a longer prepupal (cocooned 5th stadium larva) summer aestivation to cope with the extended warm temperatures in their range compared to bees natal to Utah and Washington (Sgolastra et al. 2011, Pitts-Singer et al. 2014). If Utah and Washington bees produce offspring in California during February-March almond bloom, and if they remain under ambient California conditions, they are unlikely to survive the winter because they become adults in the summer and their fat reserves are depleted before temperatures decline to initiate winter diapause (Sgolastra et al. 2011, Pitts-Singer et al. 2014).

Thus, bees from cooler climates, where the majority of commercial blue orchard bee populations are sourced, must be managed at artificial temperatures for much of the year in order to survive in a warmer climate such as in central California (Bosch et al. 2000). This difference in developmental phenology may be due to local population genetics that represent geographic variation in climatic adaptation (Bosch et al. 2000, Pitts-Singer et al. 2014). The existence of other local adaptations of these bees are unknown, such as reproductive potential or other measures of performance. Also, limited information is available about regional differences in the incidences and communities of pests and pathogens of blue orchard bees.

Additionally, no published study has yet tested the effects of mixing bees from regionally distinct localities. Preliminary research performed across two years demonstrated that crossbreed blue orchard bee offspring from California- and Utah-sourced populations matured at different rates and the emergence timing between the sexes was mismatched (Glen Trostle, personal communication). However, this work examined reproduction from one hybrid cross each year (Year 1: CA♀ × UT♂; Year 2 UT♀ × CA♂) and did not simultaneously examine a pure cross.

The consequences of mixing geographically distinct blue orchard bee populations with respect to performance and management implications is largely unknown. It is unclear if mixed-source populations are more/less likely to mate outside of their own local genotype, which may be mediated by chemical recognition cues. Furthermore, mixing geographically distinct populations could be a concern due to movement of pests and pathogens, such as chalkbrood (*Ascosphaera* spp.) and bacteria (*Wolbachia* spp.*)* that may affect mating (Breeuwer and Werren 1990, Champion de Crespingy and Wedell 2006, Hedtke et al. 2015). Controlled experiments are needed to determine if the production of subsequent generations is imperiled when subspecies or geographically distinct populations are interbred.

To examine the effects of mixing geographically distinct populations blue orchard bees on nesting and reproductive success, California- and Utah- sourced bees were paired and flown in screened field cages. The parental (P) and first filial (F1) generation were monitored to determine nest establishment, reproduction, progeny development, and progeny emergence by cross type. These results inform whether mixing geographically distinct populations of blue orchard bees, which is a likely scenario when using them for pollination of large-acreage crops, have consequences in their performance.

Materials and Methods

Study site. A monoculture plot of lacy phacelia (*Phacelia tanacetifolia* Bentham), planted and maintained by a seed-growing farm in Winters, California (36.62, -121.98), served as our study site. Large (6.1 × 6.1 × 1.8 m) screen field cages were placed overtop the phacelia to contain bees within each treatment and to provide ample and attractive floral resources (Peterson and Artz 2014) (Fig. 3-1A). At the center of each cage, one wooden nesting block was secured to two metal fence poles at approximately 1 m above ground (Fig 3-1B). The nesting block was oriented to face southeast and was sheltered by a dark blue water-resistant material (length by width by height = 22 × 20 × 26 cm) (as in Artz et al. 2014). Within each nesting block was 49 drilled tunnels (arranged in a 7 × 7 grid with cavities 15 cm in length and 7.5 mm in diameter) with paper straw inserts.

Cages were positioned in an east-west facing line, and treatments were randomly assigned to cages. The same nesting materials and screened cages were used to contain P and F1 generations during Year 1 and Year 2, respectively. The same floral resources, managed in the same way by the same farm, were also used for both nesting seasons. Year 1 of the experiment was initiated (first batch of bees released) during sweet cherry bloom in that region, 16 March 2018. Due to a later planting date and cooler winter and spring temperatures, Year 2 was initiated later, on 07 April 2019.

Floral density assessment. To assure that there were ample floral resources across all treatments, three 0.3 × 0.3 × 1 m (length by width by height) quadrats were placed in each cage and counts of open flowers and racemes were made every 3-5 days to calculate floral density throughout the experiment (all cages were assessed on the same days). Each quadrat was further subdivided into four sub-quadrats. Within each sub-quadrat, every raceme with at least one open flower was counted and the number of open flowers on 10 racemes (if available) from various heights was also counted.

The counts of all sub-quadrats were added together for each quadrat, and a mean was calculated from the three quadrats in each cage. The mean number of flowers per quadrat was then multiplied by the area of the whole cage to estimate the number of open flowers per cage. This number was divided by the number of female bees released in each cage to obtain the estimated number of flowers available per female over time. These assessments revealed that floral density was uniform across cages throughout the nesting season during Year 1 of the experiment, and that the availability of floral resources was unlikely to have limited the ability of any female to make nests, as the estimated floral density was between 5,000 to 20,000 flowers per released bee during most of the nesting period (Fig. 3-2). The same floral density data was collected in Year 2 but has not been compiled and reported here; field observations of the phacelia plot in Year 2, that was identically managed to that of Year 1, report that the onset and progression of flowering was uniform across cages.

Bee Population Crosses. Parental bees were sourced from commercial suppliers, who trapped natal nests from wildland populations (in 2017) in the Sierra Mountain foothills east of the Central Valley of California (elevation 500-1,000 m) and in the mountains surrounding Salt Lake Valley in Utah (elevation 1,700-1,900 m). These populations were managed by the suppliers and then transported to a holding facility near the study site in spring 2018, where they were stored at a wintering temperature (approx. 4°C) until the start of this experiment.

Male and female bees were paired from the same population to create the following parental (P) generation unmixed crosses: 1) CA♀ × CA♂ (CA-unmixed) and 2) UT♀ × UT♂ (UT-unmixed). We also paired males and females from reciprocal populations for the following P generation mixed-source crosses: 1) CA♀ × UT♂ (CA-mixed) and 2) UT♀ × CA♂ (UT-mixed). Each of the four P generation crosses served as a treatment for testing effects of mixing (or not) geographically distinct blue orchard bee populations on their performance in Year 1 of the experiment. These crosses resulted in first filial (F1) generation females with the following mixed and unmixed 'genotypes': 1) CACA♀, 2) CAUT♀, 3) UTUT♀, and 4) UTCA♀. Since (haploid) male bees receive only maternal input, F1 crosses resulted in either CA♂ or UT♂ genotypes, depending on the natal origin of the mother.

To simulate the downstream effects of mixing (or not) wildland-sourced populations and continuing to rear bees from this stock, mixed F1 genotype progeny from Year 1 were subjected to further mixing while unmixed genotypes were kept unmixed. Female bees from each F1 mixed genotype were paired with F1 males from both populations to create the following mixed-population crosses: 1) CAUT♀ × CA♂, 2) CAUT♀ × UT♂, 3) UTCA♀ × UT♂, and 4) UTCA♀ × CA♂. We also paired F1 unmixed genotype females with males from their respective regional population source to form the following unmixed-population crosses: 1) CACA♀ × CA♂, and 2) UTUT♀ × UT♂. Crosses from Year 2 resulted in F2 generation females and males each with mixed and unmixed genotypes. Because few bees from the CA-unmixed cross survived to emerge in spring 2019, we obtained and used female (five) male bees (eight) from the same California bee stock that were used in Year 1 to fly in cages that required unmixed CA genotypes for mating in Year 2.

Bee body measurements and paint-marking. On 15 and 16 March 2018 (Year 1), 120 female bees (60 from each source population) were temporarily removed from cold storage, excised from their cocoons, photographed and uniquely paint-marked, then held in petri dishes labeled with treatment information at standard refrigeration temperature (4°C) until released. Male cocoons (72 from each source) were selected, excised from their cocoons to verify sex, and stored in labeled petri dishes at standard refrigeration temperature (4°C) until released.

Photographs were taken with a DSLR camera and a macro-photography lens, mounted on a tripod. One at a time, chilled bees were removed from the fridge, excised from their cocoon, and forceps were used to hold bees in a modified pinning unit tray while photographs were taken (Fig. 3-3). Photographs were taken so that a mm ruler, the bee, and bee identification were all within the frame and on the same plane. The software Image-J (Rasband 1997) was used to measure intertegular space as an estimate of relative body size (Cane 1987, McCabe et al. 2021).

Immediately after the photograph was taken, each female was marked with a unique color code on the thorax with enamel paint; color-codes were used such that no two individuals had the same markings in any given cage. Color-codes and identification from the photographs were recorded, along with population source. Once bees were painted, they were stored in a labeled petri dish and returned to cold storage until release. In 2019, F1 generation bees were similarly paint-marked as in Year 1 for further experimentation in Year 2 (Table 3-1).

Bee release and nesting data collection. On 16 March 2018 (Year 1), 15 females and 18 males for each treatment (UT-unmixed, UT-mixed, CA-unmixed, and CA-mixed) were released in eight field cages (two cages per cross type). Due to post-release mortality, a second batch of bees was released to replace the dead ones on 22 March

(Table 3-1). Observations of female bees were made at each nesting block to determine nest ownership, so that maternity could be assigned to progeny (via paint marking color code). Each nesting block was observed for 15 minutes or until all uniquely marked females were observed, every 1-3 days.

Additionally, progeny production was monitored by temporarily removing paper straw inserts from the nesting block and marking nest progression using a sharpie pen. Paper straws were labeled by cage and position in the nest, and female ID if available, for later data entry. Nest progression checks were performed every 1-2 days late in the evening when foraging had ceased or early in the morning before foraging had begun. This data informed the timing of nest building and reproductive output and was paired with nest observations to associate this data female identification. Digital radiographic images (12 s exposure at 24 kVp; computed radiography high-resolution system by Faxitron X-Ray LLC, Linconshire, IL) were taken during winter management (see below) to reveal nest contents and survival to the adult stage.

An odds ratio chi-squared test was used on the to compare the likelihood of nest establishment by maternal genotype. A generalized linear mixed models was used to compare the size measurement of females (intertegular space), nest initiation time (number of days between release and first cell completed), nesting duration (number of days bees provisioned nests), reproductive output (number of cells produced), and sex ratio (proportion of female progeny) by population cross. Since only fertilized eggs result in female progeny, sex ratio is indicative of mating success. Due to low sample size, emergence timing was not statistically analyzed.

On 07 April 2019 (Year 2), surviving progeny were released in the same field cages and on the same floral resources as in Year 1. Unlike in Year 1, progeny produced during Year 2 were kept within the nest block at the study site throughout the experiment; this did not limit nesting, as there were always unoccupied nests available. Due to high mortality of the F1 generation prior to adult eclosion, the number of emerged bees available for population crosses in Year 2 was limited (see below). As such, a summary of the nesting data in Year 2 is provided, and the data was not statistically analyzed.

Bee management (rearing and emergence). Progeny produced during Year 1 (F1 generation) nesting season were removed from the study site once each nest was completed (nest entrance was closed off with soil) and moved to a sheltered and screened holding site in Foresthill, CA (39.02, -120.87). Nests containing F1 generation bees were left at ambient temperatures throughout their immature development and were periodically inspected (visually via longitudinal slice in paper straw inserts) so that the number of brood cells produced, mortality, and progression of larval stages could be recorded until mature larvae (in the fifth stadium) completed their cocoons.

Bees were transported to the Pollinating Insects Research Unit (PIRU) in Logan, Utah on 25 July 2018 and then were transferred to labeled gelatin capsules so that radiographic images could be taken every other day to document transitions from pupal to adult stages. During this period, bees were held at room temperature, approx. 20-22°C. Approximately 30 days after mean adult eclosion (14 August), bees were prepared for winter by gradually cooling rearing temperatures to 5°C (01 October) until spring 2019 (Table 3-1). Radiographic images and visual inspection of bee nests revealed that mortality during immature development (at the study site and/or holding site) was very high. Many bees died due to infestation by a parasitoid wasp (*Monodontomerus* spp.

[Hymenopera: Torymidae]), despite screened protection, and pollen balls (i.e., no egg was laid on pollen provision or bee died in the egg stage).

On 08 and 09 March 2019 (Year 2), cocooned bees were transported in a cooler with ice packs (stored in a standard refrigerator, approx. 5°C, overnight) from PIRU to a holding site nearby the study, where they were again stored at 5°C. Starting 19 March, six male bees were noticed to have emerged (in cold storage). Bees were then removed from cold storage and held at room temperature (20-22°C), and monitored daily to record the timing of emergence. Emerged bees were placed in holding containers until released, by population cross type. Females were photographed and paint marked as they emerged and then placed in holding containers by treatment. Males were pooled into one of two containers by maternal genetic input, CA or UT. All holding containers provided sufficient airflow, folded paper as a resting surface, and feeders with honey-water (1:9) that were changed every other day until released in experimental field cages.

On 26 April 2019, progeny produced during Year 2 (F2 generation) nesting season were moved to the same holding site as used in Year 1 and allowed to develop at ambient conditions. They were then transported to PIRU on 24 July and radiographic images were taken while nests were still in paper straws; here, they were held at room temperature (20-22°C). Approximately 30 days after mean adult eclosion (01 September), bees were managed for winter in a similar manner as the F1 generation, and placed at 5°C on 15 October until spring 2020 (Table 3-1). While still in cold storage, cocooned bees were moved to labeled gelatin capsules so that bees were individually contained prior to emergence. On 06 April 2020, rearing temperature of F2 generation bees was raised to 24°C to induce emergence. Starting 07 April, bees were checked daily for emergence until no bees had emerged for 5 days in a row.

Results

Body size measurements. For parental generation females, we found that UT females were about 5% larger than CA females ($F_{1,132}$ = 15.68, p = 0.0001) (Fig. 3-4).

Nest establishment and reproduction. Odds ratio chi-square test found that UT female bees were twice as likely to establish nests as CA females (χ^2 = 5.06, df = 1, p = 0.024) (Fig. 3-5A). Once bees established nests, we found no significant effect of cross type on the mean number of cells produced per female ($F_{3,45}$ = 0.44, p = 0.726) (Fig. 3-6A). There were also no significant effects of cross type on the nest initiation time ($F_{3,45}$ = 0.49, p = 0.691) (Fig. 3-6B) or nesting duration ($F_{3,45}$ = 1.37, p = 0.263) (Fig. 3-6C). For the sex ratio of progeny produced, we found a significant difference by treatment, ($F_{3,50}$ = 2.90, p = 0.0438). Both cross types with UT females produced a higher proportion of female progeny than did both cross types with CA females (Fig. 3-6D); in the post-hoc test, the comparison between CA-same and UT-same crosses just failed to meet significance (p = 0.0623).

Contrary to our results in Year 1, cross types with CA females established nest in the field at a higher rate than the cross types with UT females (Fig. 3-5B). We found no observable trends in cell production, nesting initiation time, nesting duration, and sex ratio of progeny by population cross (Fig 3-7).

Emergence. The F1 generation male progeny from all Year 1 population crosses emerged before peak female emergence; most emerged at 7°C prior to incubation at room temperature (Fig. 3-8). However, for the UT-unmixed cross, some males emerged along with females under warmer temperatures. At least one female emerged at 7°C in all population crosses except for the UT-unmixed cross. Most females emerged only after incubation at room temperature (ca. 21°C). The F2 generation progeny from Year 2 all emerged rapidly after incubation at 24°C, and peak male emergence was prior to peak female emergence (Fig 3-9).

Discussion

Year 1 comparisons of California- and Utah-sourced blue orchard bees highlight an important difference in pollinator performance by geographic origin. The significantly greater rate of nest establishment by Utah bees demonstrates that post-emergence mortality may be a concern for the smaller California bees. However, nesting periods and reproductive output on a per female basis were similar in mixed and unmixed population crosses. Year 2 summary data of first filial (F1) generation bees showed an opposing trend in nest establishment, with higher rates in the CA-genotype females; other measures of pollinator performance again showed no observable trend by population cross. Together, Year 1 and Year 2 experiments suggest that downstream effects of mixing geographically distinct populations are not a concern, but that parental generations may vary in their initial performance as pollinators.

Emergence of both F1 and F2 generation bees did not show any mismatch in the natural temporal pattern by sex as a consequence of mixing populations. However, high mortality of reared progeny due to parasitism resulted in low sample sizes for the F1 and second filial (F2) generations. Thus, these effects should be further investigation. It is important to determine if mixing early-flying populations (e.g., California) with late-flying populations (e.g., Utah), and/or their translocation to this novel environment, may cause a disruption in male and female emergence timing as this could be detrimental to mating success.

The lower nest establishment of CA-sourced females in Year 1 could be attributed to their smaller size. A study by Bosch and Vicens (2006) similarly found that small bees were less likely to establish at provided nesting sites than large females, in a closely related managed species (the European orchard bee, *Osmia cornuta*). Other studies on the blue and European orchard bees have found higher overwintering mortality in smaller bees than in their larger counterparts (Tepedino and Torchio 1982, Bosch and Kemp 2004). Bosch and Kemp (2004) additionally found that smaller bees had shorter post-emergence longevity. These studies together, along with results from Chapter III (this thesis) suggest that low nest establishment of smaller females is due to mortality during the pre-nesting period, likely due to reduced fat bodies upon emergence, and not to greater dispersal from release sites.

Interestingly, bee size did not appear to impact pollinator performance or reproductive potential of the females that did survive to establish nests in the provided materials, in this study or in the previously mentioned works. Specifically, no observed effect of body size was found on fecundity or sex ratio of progeny (Tepedio and Torchio 1982), or on emergence timing (Bosch and Kemp 2004). So, while bee body size may not be a predictor of reproductive capacity on a per female basis, it is likely that the increased rate of nest establishment and overwintering survival of larger females would result in a net gain in the managed population from one pollination season to the next. A study by Tepedino et al. (1984) has concluded that body size has low heritability in this species, and thus, a breeding program to select for increased size would be unfruitful (Tepedino et al. 1984). However, using parental populations with large body size, and ensuring that abundant floral resources are available during the entire nesting period could be a viable strategy for optimizing bee size since larger pollen provisions typically results in larger sized progeny (Klostermeyer et al 1973, Bosch and Vicens 2002).

This is the first reported study to pair parental generation blue orchard bees from geographically distinct populations in a controlled experiment to examine its effects on nesting behavior and reproductive output. Even though Year 2 experiments could not be statistically analyzed due to low sample size, these studies provide new insight into the consequences of mixing blue orchard bee populations. Specifically, it does not appear that the production of subsequent generations is imperiled when geographically populations are interbred, but parental populations may vary in their rates of nest establishment.

Suppliers and managers need more information about regional variation blue orchard bee populations to supply growers and other customers with disease- and pest-free bees, to manage their bees for optimal performance, and to maintain populations in numbers large enough for profitable business and pollination in conventional farming operations. More research is needed to determine recommended stocking rates for specific bee stock for use in particular crops and growing regions, and whether certain populations show favorable traits for propagation in orchard settings. Nonetheless, it is wise to limit the movement and mixing of geographically distinct populations in order to reduce the spread of pests and diseases, and to maintain population-level genetic differences that may play a currently unknown role in their performance as pollinators.

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Tables

Table 3-1

Timeline of Experimental Events Crossing California- and Utah-sourced Bees

Date	**Event**
15-16 Mar 2018	Parental generation bees were temporarily removed from cold storage (4°C) and excised from their cocoons to verify sex and organize bees by treatment in labeled petri dishes. Females were additionally photographed and uniquely paint-marked.
16 Mar 2018	For each population cross (CA♀ × CA♂, CA♀ × UT♂, UT♀ × UT♂, and UT♀ × CA♂), 15 females and 18 males were released in eight field cages (two cages per cross) in Winters, California.
19 Mar 2018	Nest progression checks were performed every 1-2 days and nests were observed every 1-3 days, during the nesting season (bees were left undisturbed for the first three days after release).
22 Mar 2018	A second batch of marked females (two CA-sourced and two UT-sourced) was released to compensate for post-emergence mortality.
26 Mar 2018	A third batch of marked females (four UT-sourced) was released.
18 Apr 2018	Year 1 nesting season was terminated; any remaining bees were collected and field cages were disassembled.
25 Jul 2018	All progenies (F1 generation) were transported to the Pollinating Insects Research Unit (PIRU) in Logan, UT so that radiographic images could be taken to document transitions from pupal to adult stages, and bees managed until spring 2019. Bees were previously held at outdoor ambient temperatures at a holding site near the study site and then at room temperature (20-22°C) at PIRU.
14 Sep 2018	All bees, now cocooned adults, were transferred to a test chamber, and held at 15°C for the first in a stepwise process to prepare bees for cold wintering (diapause) temperature.
24 Sep 2018	Rearing temperature was lowered to 10°C.
01 Oct 2018	Rearing temperature was lowered to 5°C and held at this wintering (diapause) temperature until spring.
08-09 Mar 2019	Cocooned bees were transported in a cooler with ice packs (stored in a standard refrigerator, approx. 5°C, overnight) from PIRU to a holding site nearby the study, and again stored at 5°C.
19 Mar 2019	Bees were moved to room temperature (20-22°C), and monitored daily to record emergence timing. F1 generation bees were similarly prepared for experimentation as in Year 1. Once emerged, they placed in holding containers by cross type until released.
07 Apr 2019	All bees were released in field cages to initiate Year 2 nesting season. Bees were left undisturbed for three days, and then nest progression checks were performed every 1-2 days and nests were observed every 1-3 days, during the nesting season.

26 Apr 2019	Year 2 nesting season was terminated. Progeny (F2 generation) were moved to the same holding site as used in Year 1 and allowed to develop at ambient conditions.
01 Oct 2019	All bees, now cocooned adults, were transferred to a test chamber and held at 19°C for the first in a stepwise process to prepare bees for cold wintering temperature.
08 Oct 2019	Rearing temperature was lowered to 11°C.
15 Oct 2019	Rearing temperature was lowered to 5°C and held at this wintering (diapause) temperature until spring.
06 Apr 2020	Rearing temperature was raised to 24°C to induce emergence, and bees were checked daily to record emergence timing.

Note. Parental generations of blue orchard bees sourced from California and Utah wildland populations were crossed in screened field cages with lacy phacelia available as forage in spring 2018 (Year 1). The resulting first filial (F1) generation progenies from cross-bred and unmixed populations were reared, using standard management protocols, and released in the same field cages and on the sage forage as in Year 1 the following spring (Year 2). The resulting second (F2) generation progenies from Year 2 were reared and emerged the following spring.

Figures

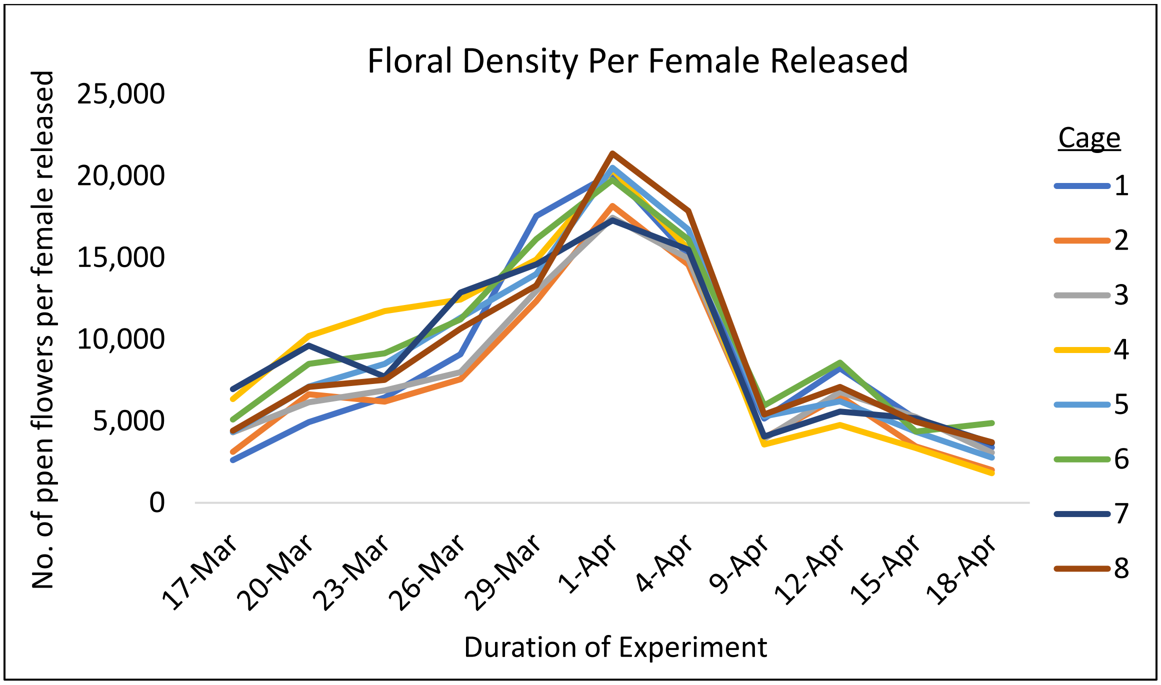
Figure 3-1

Screened Field Cages and Nesting Materials

Note. (A) Screened field cages used to contain blue orchard bees in a controlled experiment examining population crosses. Lacy phacelia provided ample and attractive floral resources for bee nesting (Peterson and Artz 2014). (B) Nesting materials (wooden block containing nesting cavities lined by paper straws and housed by a plastic shelter) were placed in each cage to monitor nests and nesting females.

Figure 3-2

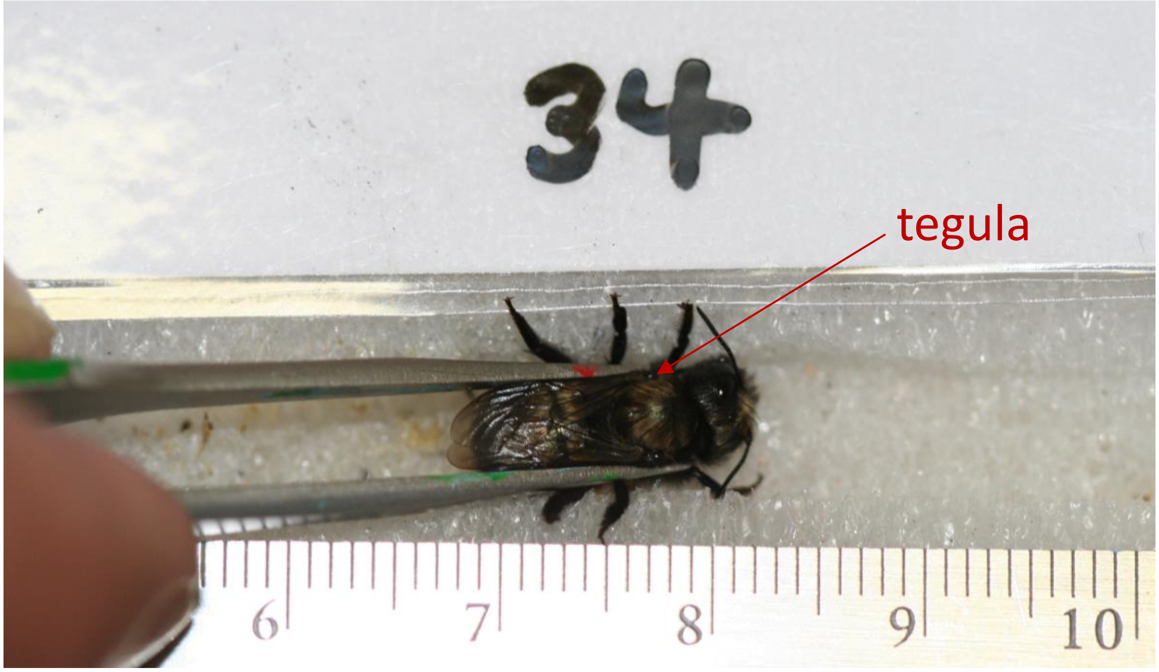
Estimated Floral Density



Note. Lacy phacelia was used to support blue orchard bees flown in field cages; bees were sourced from California and Utah wildland populations to examine effects of crossbreeding in spring 2018.

Figure 3-3

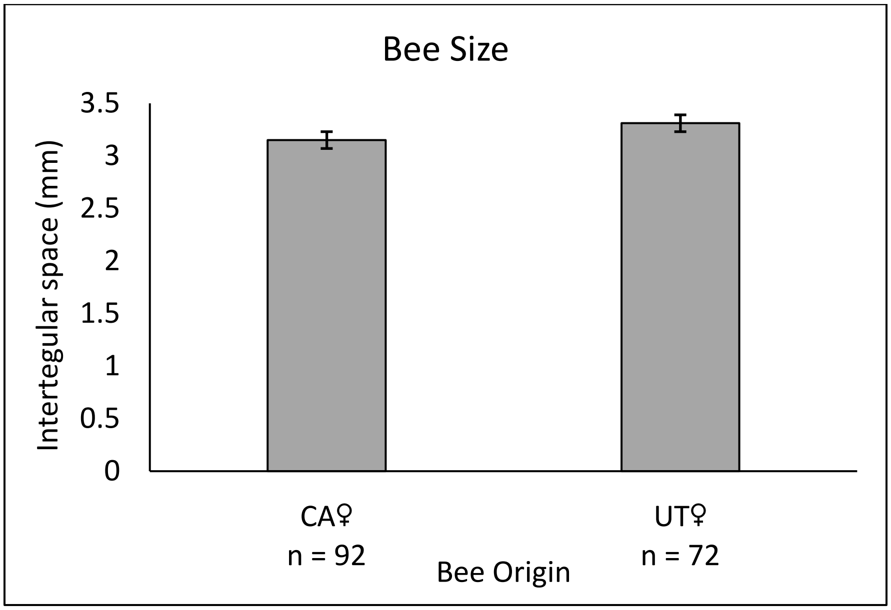
Intertegular Space Measurements for Estimating Body Size



Note. Blue orchard bee females from California- and Utah-sourced populations were photographed to compare body size. The software Image-J (Rasband 1997) was used to measure intertegular space as an estimate of relative body size (Cane 1987, McCabe et al. 2021).

Figure 3-4

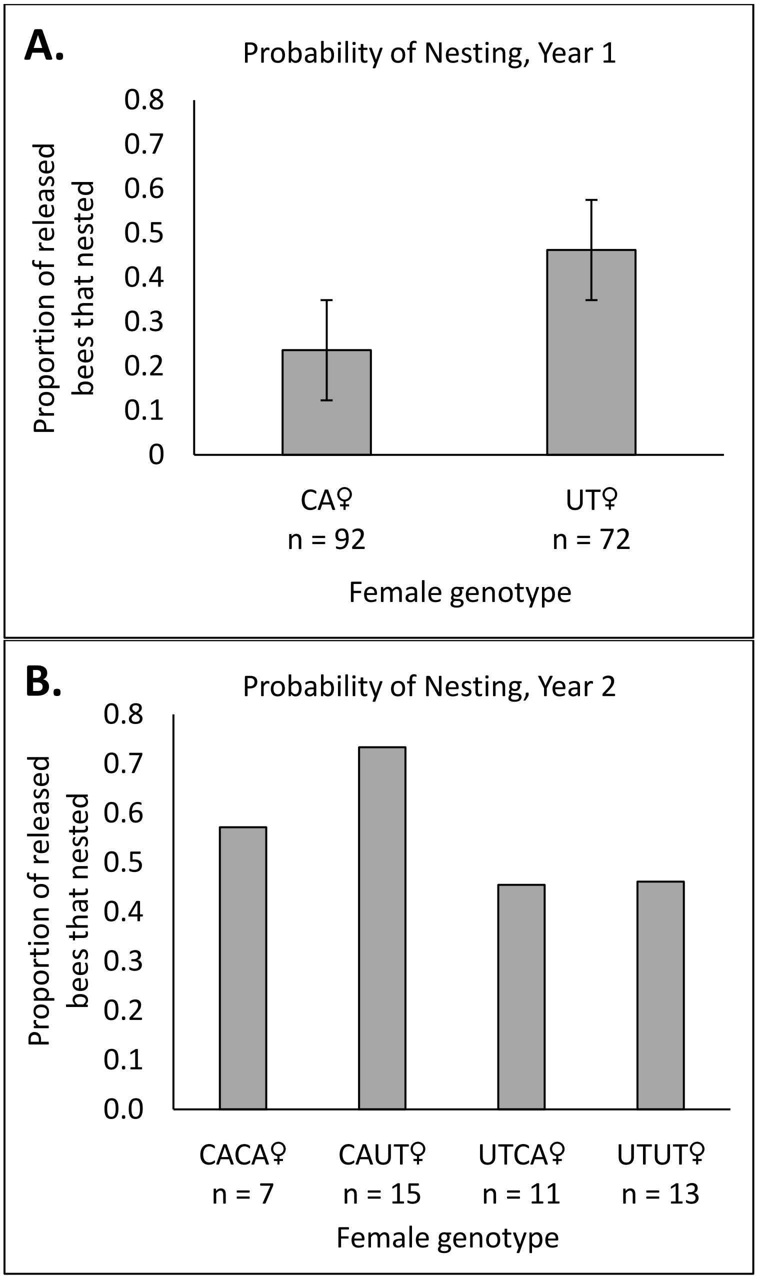
Comparison of Female Body Size by Natal Origin



Note. By parental genotype, size of female blue orchard bees. High quality photographs of bees were taken using a mount and ruler for scale and images were analyzed using ImageJ software. Intertegular space was used as a proxy for body size (Cane 1987, McCabe et al. 2021). The sample size (n) is reported on the x-axis.

Figure 3-5

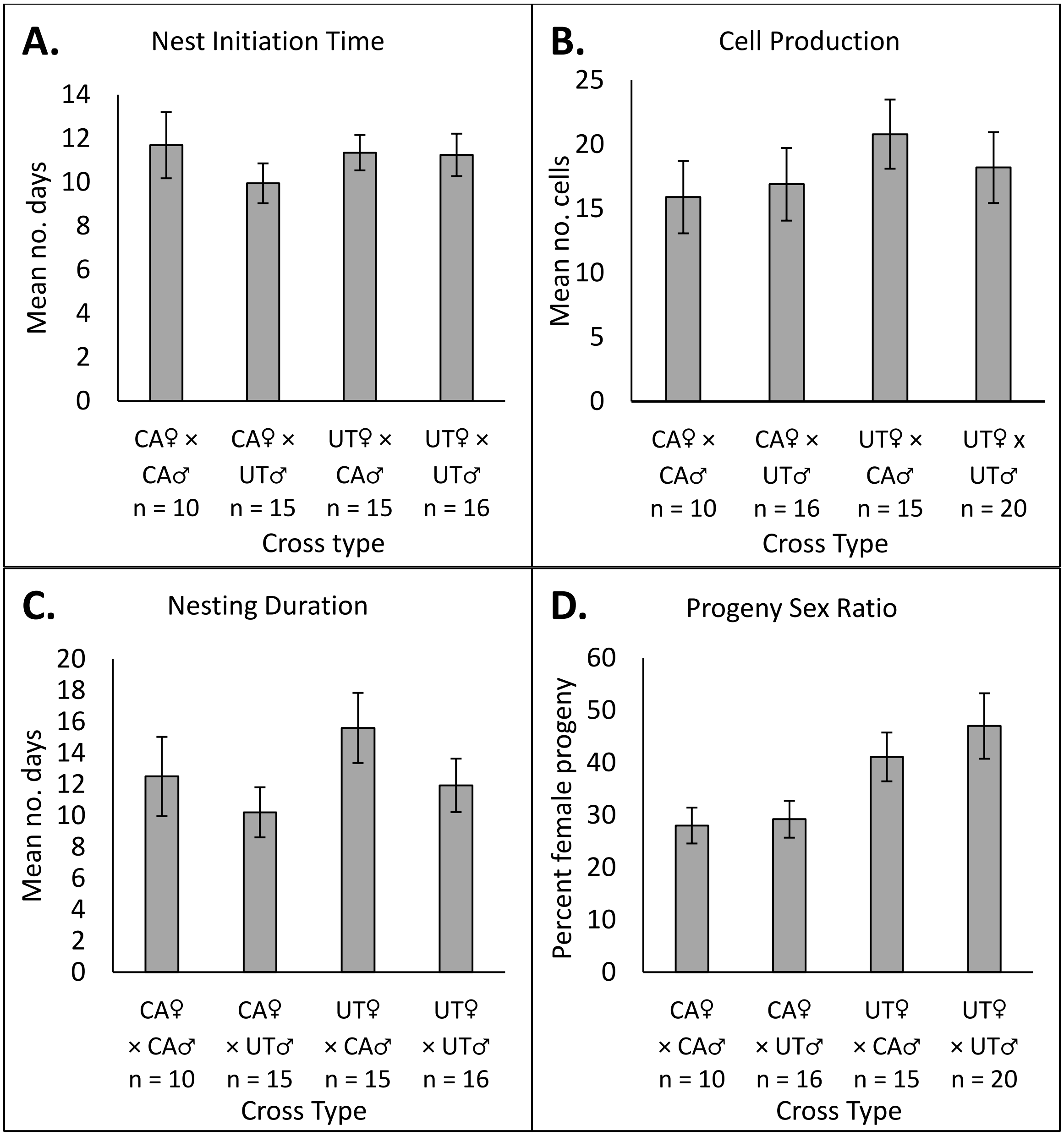
Nest Establishment of P and F1 Generation Females



Note. By (A) parental generation and (B) first filial generation female genotype, the proportion of released blue orchard bees that nested in field screened cages with abundant floral resources. The sample size (n) is reported on the x-axis.

Figure 3-6

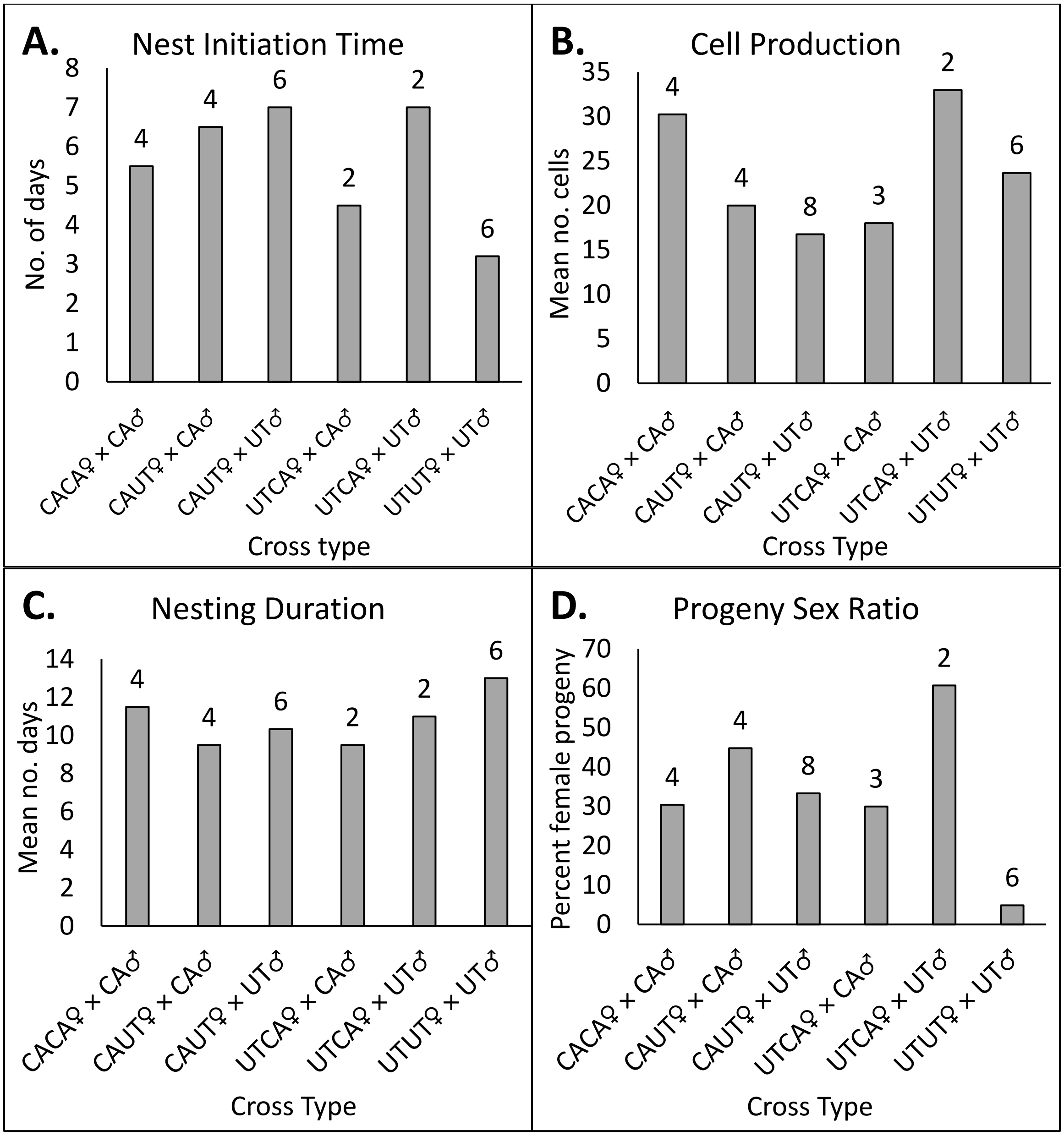
Comparisons of Pollinator Performance by P Generation Females



Note. For the parental generation of California blue orchard bees released in field cages with abundant floral resources (phacelia), comparisons of pollinator performance by (A) nest initiation time (mean number of days between release and first cell completed per female), (B) reproductive output (mean number of cells produced per female), (C) nesting duration (mean number of days spent provisioned nests per female), (D) and sex ratio (proportion of female progeny per adult female). The sample size (n) is reported on the x-axis.

Figure 3-7

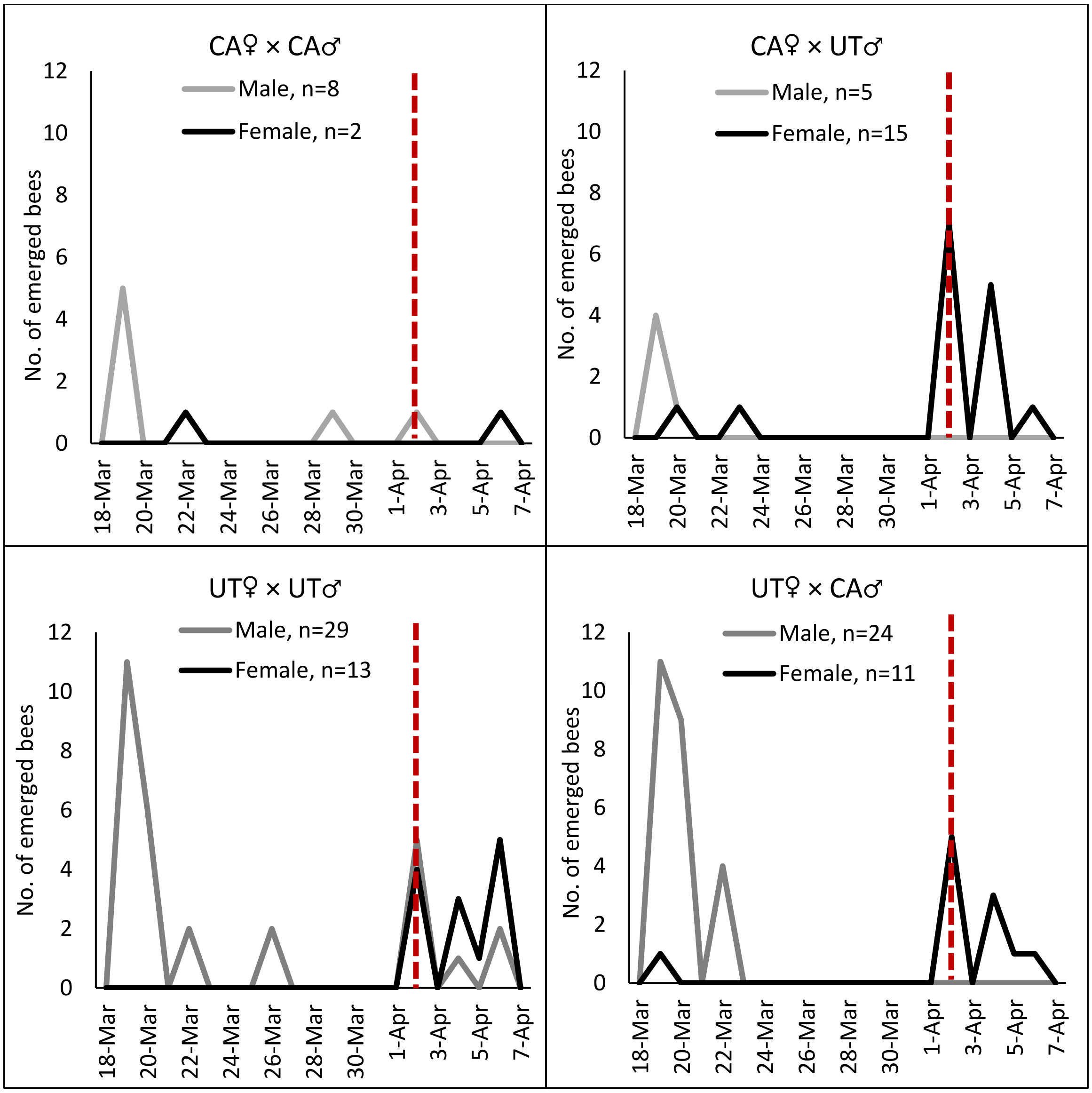
Comparisons of Pollinator Performance by F1 Generation Females



Note. For blue orchard bees released in field cages with abundant floral resources (phacelia), comparisons of (A) nest initiation time (mean number of days between release and first cell completed per female), (B) reproductive output (mean number of cells produced per female), (C) nesting duration (mean number of days spent provisioned nests per female), and (D) sex ratio (proportion of female progeny per adult female). These F1 generation bees were the progenies of California- and Utah-sourced populations that were cross-bred (P generation crosses = CA♀ × UT♂ and UT♀ × CA♂) or unmixed (P generation crosses = CA♀ × CA♂, UT♀ × UT♂). The sample size is reported at the top of each bar.

Figure 3-8

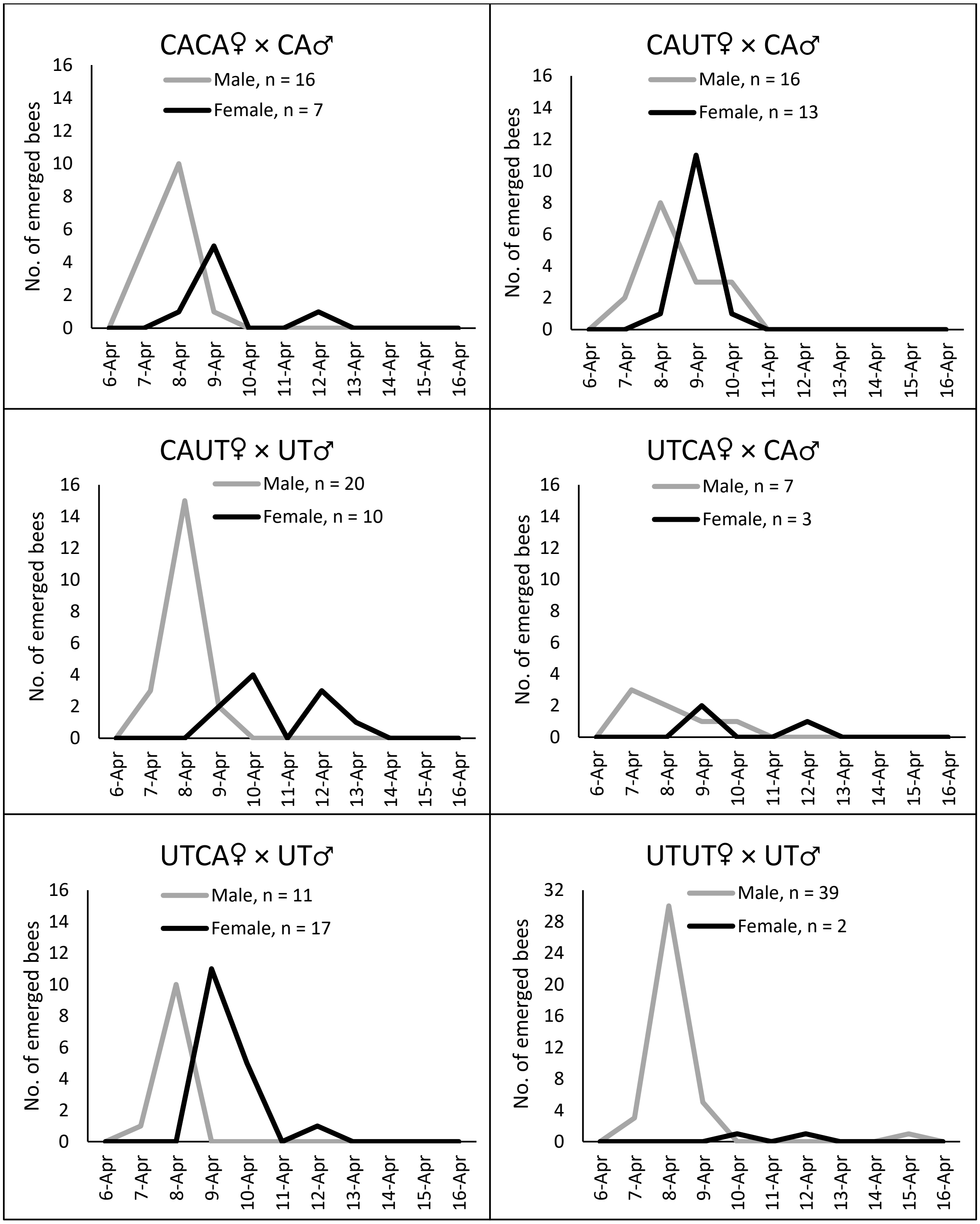
Emergence Timing of F1 Generation Bees



Note. By parental generation cross type, emergence of F1 generation blue orchard bees in spring 2019. Bees were stored at 5°C over winter and induced to emerge; on 04 February, the storage temperature was raised to 7°C, and starting on 02 April, bees were incubated at room temperature (as indicated by the vertical dashed line).

Figure 3-9

Emergence Timing of F2 Generation Bees



Note. By F1 generation cross type, emergence of F2 generation blue orchard bees in spring 2020. Bees were stored at 4°C over winter, and on 06 April (the first date on the x-axis) bees were incubated at 24°C.

GENERAL SUMMARY AND CONCLUSION

General summary

For economically viable and environmentally sustainable crop production, research on and commercial development of alternative (non-*Apis* spp.) pollinators, as well as the habitats that sustain them, should be incentivized. Mason bees (*Osmia* spp.) and other cavity-nesting bees in the family Megachilidae have proven particularly useful for the production of many pollinator-dependent crops for which honey bees are poor pollinators or are in poor supply during crop bloom (James and Pitts-Singer 2008, Mader et al. 2010, Peterson and Artz 2014). The blue orchard bee (*Osmia lignaria*) is North America's most agriculturally important native mason bee as a pollinator of spring-blooming specialty crops such as almond, cherry, pear, apple, and plum (Torchio 1976, 1981, 1982, Bosch and Kemp 1999, 2001, Bosch et al. 2006, Pitts-Singer et al. 2018).

Solitary bees are typically managed by growers (unlike honey bees in which growers pay beekeepers a set fee) for pollinating their crops. Mass-rearing often involves subjecting bees to controlled (artificial) temperatures in order to manipulate the timing of life cycle events for their effective utilization (Bosch and Kemp 2001, Bosch et al. 2008 Morales-Ramos et al. 2022). For example, established cold winter storage and warm spring incubation practices are often used to synchronize bee activity with crop bloom. Although the natural activity period of adult blue orchard bees overlaps with the bloom time of many crops, controlling emergence and subsequent nesting allowing growers and bee managers to maximize both pollination (and in turn, fruit set) and bee reproduction.

Managed blue orchard bees are often used as pollinators in a different geographic location from where they are sourced, and differences in the timing of development between populations, in ways that impact their management, have recently been identified (Sgolastra et al. 2012, Pitts-Singer et al. 2014). Populations from northern Utah, where the bee was originally domesticated, have been the most well-studied and employed as pollinators. Much about how other populations differ in their development, reproduction, and nesting behavior is largely unknown. In the studies reported here, I compared developmental biology and post-emergence performance of blue orchard bees sourced from Utah with bees sourced from other, geographically distinct locations in the western United States.

In my first chapter, I examined nest establishment of blue orchard bees sourced from two locations, the foothills of the Sierra Nevada Mountain range in California and the Rocky Mountain range in northern Utah and southern Idaho, when used as pollinators in both source and reciprocal states. I observed paint-marked bees at nesting sites in a central orchard section to evaluate female retention and at distant nesting sites to compare female dispersal.

In March-blooming California sweet cherry orchards, I found a statistically significant difference in female retention by geographic origin; over twice as many UT-sourced females were observed at center orchard sites than were CA-sourced. In May-blooming tart cherry orchards, no statistically significant differences in nest establishment were found at center or distant nesting sites between the same two populations. Bees from both populations performed better in Utah orchards. The presence or absence of unmarked bees revealed that local (wild and/or managed) bees were abundant in Utah orchards, but were likely absent in California orchards.

In my second chapter, I reared bees natal to northern Utah and western

Washington in laboratory incubators under two prescribed thermal regimens, 1) constant temperatures used to manage bees through all life stages, and 2) hourly fluctuating temperatures programmed to mimic the natural daily (24 h) thermal cycles of the nearest commercial cherry-growing region.

I found that blue orchard bees sourced from Utah and Washington differed in their survival and developmental biology when reared at a shared, constant thermal regimen (26°C); immature development was shorter and mortality higher for the Washington bees. Bees from both populations emerged quickly upon incubation in spring. All bees reared at the constant regimen had increased survival and shortened egg-adult development periods compared to bees reared at natural temperatures. At natural temperatures, high mortality coincided with the hottest maximum temperatures and late life stages (prepupa-adult). Female bees from both populations emerged in synchrony with local crop bloom at natural temperatures, but the emergence period was prolonged compared to the constant thermal regimen.

In my third chapter, I flew California- and Utah-sourced blue orchard bees in field cages with abundant floral resources to determine the effects of mixing these populations on post-emergent performance, progeny development, and progeny emergence. I then flew surviving progeny in the same field cages the following year to simulate the downstream effects of mixing (or not) starting populations and continuing to rear bees from this stock. I monitored nesting behavior and reproductive output of mixed and unmixed populations of parental and first filial generation bees, and emergence of the F1 and second filial generations.

In starting populations, I found that blue orchard bees sourced from UT were twice as likely to establish nests than CA bees within our screened field cages. The smaller body size of CA bees is proposed to be a likely cause for the observed variation in nest establishment. Of the bees that established nests in the provided materials, I found no statistically significant differences in reproductive output, time to initiate nest building, or the duration of nesting by cross type. The progeny of all four population cross maintained the natural temporal pattern of emergence by sex (males before females) the following spring. In Year 2, I found no observable trends in the same metrics of pollinator performance by the first filial generation. The second filial generation of bees also did not show any mismatch in male-female emergence timing from population crosses.

The overarching goal of this work was to investigate how western populations of blue orchard bees differ in ways that may affect their management for pollination services. Bee managers need to understand the potential consequences of bee origin and their management practices on pollinator performance and reproduction. A better understanding of blue orchard bee ecophysiology throughout its range will further the development of best management practices for making decisions about using bees from various ecoregions according to the place and season of crop bloom.

Conclusions

My research has gleaned new insight into differences between geographically distinct blue orchard bee populations that impact their performance as managed pollinators. Studies such as these are the first step in identifying how suitable various populations are for commercial management. I demonstrated that nest establishment in orchards may vary by bee origin and crop. Bee populations are not necessarily best suited for pollination within their natal regions. Geographic origin and management history may play as much of a role in bee performance as does crop type and location, the timing of bloom, and agricultural practices. Due to the gregarious nesting habit of blue orchard bees, the presence of local bee populations can increase nesting success and reproductive output of deployed populations, and thus, should be a considering factor for deciding on the stocking rate of a particular crop.

My work investigating thermal effects on geographically distinct populations has provided supporting evidence that developmental biology has a genetic basis, given the differences in survival and development of Utah- and Washington-sourced bees I detected when reared at the same, constant temperatures. This study also found evidence to suggest that bees originating from montane habitats suffer from the warmer climatic conditions of lower altitudes where crops are cultivated, even when sourced within the same geographic region. To maximize blue orchard bee survival and performance as commercial pollinators, exposure to extreme summer temperatures should be negated and stocks from distinct geographic locations should be kept as separate cohorts for management.

In a novel three-year experiment pairing blue orchard bees from California- and Utah-sourced populations, I found that parental generation bees may vary in their initial performance as pollinators but that downstream effects of mixing populations are likely not detrimental. These results help alleviate concerns over mating, nesting, and reproductive success for bee managers and orchardists who employ bees from various sources. My findings from these controlled crosses and the in-orchard experiments suggests that lower nest establishment of smaller California-sourced females is likely due to high mortality after emergence (i.e., pre-nesting period), likely due to reduced fat bodies upon emergence, and an increased propensity to disperse from release sites. Using parental populations with large body size and ensuring that abundant floral resources are available during the entire nesting period could be a viable strategy for optimizing pollinator performance.

Comparing geographically distinct populations is the first step in determining which populations demonstrate characteristics that are beneficial for commercial management. Suppliers and managers need more information about regional variation blue orchard bee populations to manage their bees for optimal performance, and to maintain populations in numbers large enough for profitable business and pollination in conventional farming operations. More research is needed to determine recommended stocking rates for specific bee stock for use in particular crops and growing regions. Nonetheless, it is wise to limit the movement and mixing of geographically distinct populations in order to reduce the spread of pests and diseases, and to maintain population-level genetic differences that may play a currently unknown role in their performance as pollinators.

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