Review of Capacity for Welfare at Different Life Stages

RESEARCH QUESTIONS

Are there different capacities for welfare {based on the basic requirements for life on page 3}?

a. Between life stages
    i. Immature
        1. Larval instars
            a. Hemimetabola
            b. Holometabola
    ii. Adult
b. Between species:
    i. Mealworm (Coleoptera: Tenebrionidae) - *Tenebrio molitor*
    ii. Soldier fly (Diptera: Stratiomyidae) - *Hermetia illucens*
    iii. Honey bee (Hymenoptera: Apidae) - *Apis mellifera*
    iv. Cricket (Orthoptera: Gryllidae) - *Archeta domesticus* or *Gryllodes sigillatus*
    v. Silkworm (Lepidoptera: Bombycidae) - *Bombyx mori*

Link to ontogeny & behavior traits table
Link to biomarker traits table

I. **Basic Traits of Life** (Next Generation Science Standards)
    A. Order (physical)

1. What are the abnormalities associated with the restricted gene pool, etc of reared invertebrates?
2. What degree of plasticity is represented in commercial versus wild populations?

B. Sensitivity/response to environment/stimuli
   1. Is there a stimuli that would not be present in wild groups to keep in mind?
      a) What is the difference between larval stages?
   2. What about the cognitive/learning capacity?
      a) Partially represented in biomarker data
   3. **TRAITS TO INVESTIGATE: cognitive/learning capacity, sentience data**

C. Reproduction
   1. For larvally-reared taxa, is reproduction enabled?
      a) If no, the capacity for reproduction would be 0

D. Growth/development
   1. How are growth and development affected over time?
      a) Triggering of hormones related to photoperiod/temperature of rearing conditions?
      b) Fill in gaps for biomarker data
   2. **TRAITS TO INVESTIGATE: development of sensory system, time in each stage**

E. Regulation of functions
   1. How are they disrupted by nutrition, environment related to rearing?
   2. **TRAITS TO INVESTIGATE: behaviors exhibited in each stage (feeding, movement, etc), ontogeny of neurological/endocrinological/morphological traits (hormonal cascades and responses)**

F. Homeostasis
   1. How are the external conditions from farming influencing homeostasis?
   2. What are the pathogens that individuals are exposed to in the wild versus reared populations?
   3. **TRAITS TO INVESTIGATE: responses to external changes (behaviors, movements), life history differences from rearing**

G. Energy processing
   1. Nutrition and waste capacity?
   2. **TRAITS TO INVESTIGATE: diet, foraging**

H. Survival for adaptive capacity
   1. If there is never any possibility for reproduction, this condition is not met

LIFE TRAIT SYNTHESIS METHOD (building on list of vital traits from the previous page)

Vital raits are the vital biological, ecological, and evolutionary characteristics of an organism that comprise an organism's species-specific requirements for survival (Kearney et al., 2010). The combination of these traits can demonstrate how a species fulfills a specific niche within an environment, period of time, or in relation to other organisms (Ackerly et al., 2006). Traits can be represented by qualitative or quantitative data and can be sourced through the metadata of a range

of other studies. Vital trait categories can be used in comparative research for identifying differences in the traits themselves as well as knowledge gaps between groups, particularly when overall data may be patchy and understudied in direct trait comparison between groups or life stages. Due to phylogenetic conservatism, some degree of species-specific traits can be a proxy for unexamined, but closely related species due to shared evolutionary history and similar degrees of plasticity between environments. Through an assessment of vital traits at the species-level, our aims are to figure out how to score the different species on their capacity for both possessing these traits at different life stages and having the potential resources to exhibit or access them over stages of development, in the hope to quantify and synthesize the differences between stages and taxa.

We would consider meeting needs for their welfare to be the ability to meet these basic components required for living organisms. While most people would assume that capacity for welfare is solely tied to cognitive and neurological capacity (as an organism perceiving an adequate or good life), there are basic physiological traits and morphological structures that are required to enable a capacity. These traits and structures develop between life stages (the ontogeny of an organism). Having more data points to compare enables us to make a more concrete assessment of the differences between species and stages. If we can compare the ability to meet these requirements between species/stages, we may be able to a. identify the gaps in our knowledge of the experience of these requirements at the different stages and b. rank the capacity between both life stages and species. If we assume that scores are based on trait presence, we can compare scores between stages and taxa to identify trait and knowledge gaps.

POTENTIAL SCORING SYSTEM

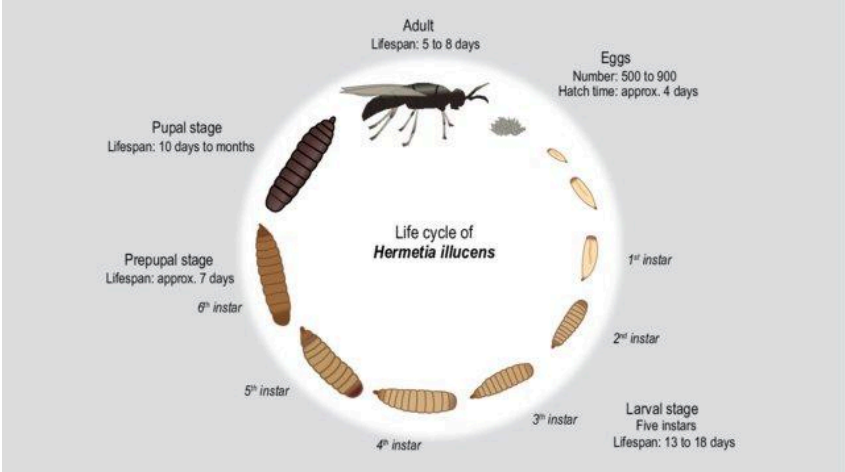
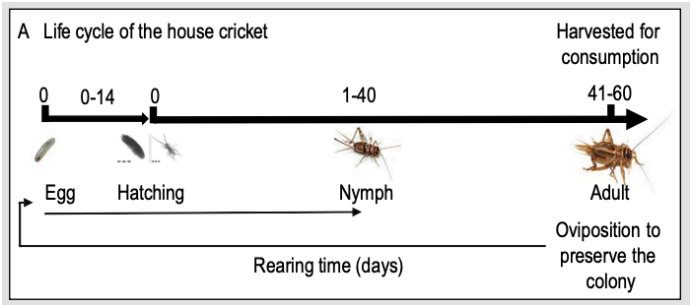
LIFE TRAIT SYNTHESIS TABLE

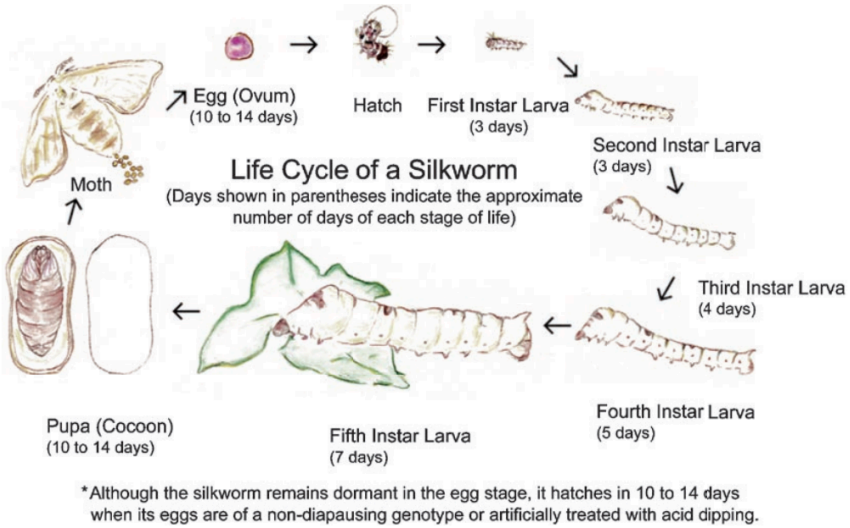
In this table, evidence of each specific life trait is grouped by source for that trait (i.e. Invertebrate Sentience Table, Capacity for Welfare Table, or both) and delineated by if it has been found to be present/examined/researched in each life stage in question (larval or adult primarily).

II. LIFE STAGE/LIFE CYCLE BY SPECIES

SPECIES	NATURAL	REARED (to consider if timing is different)
mealworm	(holometabolous) “one female of T. molitor produces 160 eggs in her life (3 months). Furthermore, the maturation period is short; T. molitor reaches adulthood in 10 weeks”(Oonincx & Boer, 2012) **- check to see if this is representative of all wild populations** *High degree of variation in life cycle related to temperature/oxygen/nutrition access, larval instars have highly variable lengths of time (Park, Jong Bin et al., 2014) **160 day life cycle, with 14 to 20 instars** (Monceau et al., 2017)	Friederich U, Volland W (2004) Breeding Food Animals: Live Food for Vivarium Animals; Friederich U, Volland W, editors. Malabar, Florida: Krieger publishing company. 178pp. Overview of conditions for mass rearing (Ribeiro et al., 2018)
honey bee	(holometabolous) “Most colonies (97%) survive summers, but only 23% of founder (first-year) colonies and 84% of established colonies survive winters. Established colonies have a mean lifespan of 5–6 years and most (87%) have a queen turnover (probably by swarming) each summer.”(Seeley, 2017) The expected life span of bees initiating foraging (ex) was 6.3 days and the average life span of foragers was 6.760.19 days.(Dukas, 2008)	winter/summer workers (Amdam & Omholt, 2002)

	 (“The Complex Life of the Honey Bee | Purdue Pesticide Programs,” 2017) **~30 day life cycle with 5 larval stages** (“The Complex Life of the Honey Bee | Purdue Pesticide Programs,” 2017)	
soldier fly	(holometabolous) “females lay a single egg batch and die shortly thereafter (Tomberlin et al. 2002), size is potentially more important for fitness of the black soldier fly than is longevity” (Tomberlin et al., 2009)	“41.6 days overall from neonate larvae to adult” (Tomberlin et al., 2009)

	 (Lievens et al., 2021) **~45 day life cycle with 6 larval instars (Lievens et al., 2021)**	
cricket	(hemimetabolous) *Archeta domesticus* - "A rearing temperature of 32°C will give a life cycle from egg to adult of 6-7 weeks. Under optimum conditions, 500-mg (mean wt) crickets are produced. There are 10 molts in the course of development." (Patton, 1978)  (Fernandez-Cassi et al., 2019) **~40 day life cycle with 10 larval instars (Patton, 1978)**	

silkworm	(holometabolous)  (Banno et al., 2010) **~45 day life cycle with 5 larval instars (Banno et al., 2010)**	<- note the acid treatment or non-diapausing genotype

III. ONTOGENY

A. Sensory System Ontogeny for groups of interest

1. General Insect development/expression patterns

a) Expansion of understanding of *hox* impression for general body plan development and differentiation expanded from what had previously been known primarily from *Drosophila.* (Janssen et al., 2018)

b) Neuroblast development review (includes holo- & hemimetabolous patterns), talks in terms of *Drosophila* homologous structure/terminology. (Urbach et al., 2003)

(1) "basic aspects of the spatial and temporal development of the embryonic brain neuroblast pattern are conserved among insects. Furthermore, we compare the number and proliferation patterns of neuroblasts related to major neuropil structures such as mushroom bodies, central complex, and antennal lobe."

(2) This study has really excellent figures

c) Classical paper in development patterns (neural and imaginal discs, etc) between insect taxa, specifically featuring research on *Apis* and *Tenebrio.* (Palka, 1979)

d) Classic review of the neurons and sensilla development in Insecta, features some information on different castes of *Apis mellifera,* as well as *Bombyx mori.* (Rospars, 1988)

e) The mushroom bodies and central complex (particularly the less-understood fan-shaped component that integrates information to motor targets) are the neural components responsible for nociception in insects, as well as other complex tasks (Adamo, 2019)

(1) Note that while these components cannot be directly comparable to vertebrate pain experience, but can influence behavioral and motor-related outcomes

(2) *"Although larval insects show sensitisation of nociception after damage that resembles both hyperalgesia (Walters et al., 2001; Babcock et al. 2009) and allodynia (Babcock et al. 2009; McMackin et al. 2016), adult insects of a number of species will walk on damaged limbs (Eisemann et al. 1984)."*

(3) Adamo reminds us that there are a lot of input neurons, but relatively few output neurons and no direct connections between them, so little integration is possible and instead these two neural structures are functioning in parallel with one another

2. Mealworm (Coleoptera: Tenebrionidae) - *Tenebrio molitor*

a) Personality and behavior is not consistent between stages (Monceau et al., 2017)

(1) "larval personality and behavioural syndrome did not predict adult behaviour. We suggest that the complete reorganization during metamorphosis may have profound effect on the

behaviour of the beetles. These results challenge the established common thought that personality should persist along an individual lifespan."

b) Responses to environment change significantly throughout the life cycle, causing overall changes to the expression and plasticity of an organism (Belén Arias et al., 2011)
    (1) "We conclude that chill-coma, recovery time and Hsp70 gene expression are plastic in response to a thermal environment but also change significantly their responses depending on the ontogenetic stage, implying that the response of adult individuals is linked to early stages of the life-cycle."
c) Neuronal development across life stages (larvae, pupa, and adult) indicates that crustacean cardioactive peptide (CCAP) immunoreactive neurons are present throughout all of metamorphosis (Breidbach & Dircksen, 1991)
    (1) "The occurrence of CCAP-IR in these structures and in ontogenetically persistent putative interneurons and peripherally projecting neurons provide further support for the suggestion that CCAP might play an important role as a neurohormone and/or neurotransmitter in central information processing and on peripheral targets (Dircksen and Keller 1988; Dircksen et al. 1991)."
d) Epigenetics (genotypic and phenotypic traits by environment) demonstrated in life history responses to rearing temperature (Prokkola et al., 2013)
    (1) "The genetic correlations between temperatures showed that there are genotype-by-environment interactions in the development times of mealworm beetles across temperatures that they can encounter in their natural habitats."
e) Neuroblast development and Engrailed gene expression in embryonic development (Urbach et al., 2003)
    (1) " A subset of five neuroblasts, among them the two progenitors of the mushroom bodies and two progenitors of the larval antennal lobe, are morphologically identifiable by their larger size."
f) Quantification of the subesophageal ganglion neurons throughout development shows that these are homologous throughout much of the insect phylogeny (Breidbach & Urbach, 1996)
    (1) "It has been shown that not only the principal structural characteristics but also the finer details of the side projection patterns of the structurally homologous neurons (Kutsch and Breidbach, 1994) are phylogenetically conserved in the subesophageal neuromers of different insect species, irrespective of different functional characteristics of these ganglions (Breidbach, 1991)"

g) Midbrain development has been examined in the larval stages, finding homologous structure and development to that of *D. melanogaster* (Wegerhoff & Breidbach, 1992)

3. **Soldier fly (Diptera: Stratiomyidae) - *Hermetia illucens***
    a) Mathematical model for life history and growth of the larva over time is affected by moisture, temperature, and feeding (Padmanabha et al., 2020)
        (1) “The dynamic model, proposed and validated in this work, consistently explained: the change of larval dry mass over time; transition of development phase and its effect on growth; and influence of external factors on the larval growth and development.”
    b) Antennal and sensory system development (mechano- and chemoreception) over time can be tracked between sexes and development (Birrell, 2018)
        (1) Population density at different life stages can lead to trait selection that is beneficial for survival of the larval stage, but leading to changes in the sensory capacity throughout the life cycle
    c) Food volatile behavioral choice test of larvae and adults, diet effects on behavior and development (Loreto 2018 Thesis)
        (1) Has some good background
    d) Light sensitivity measurements were measured by TEM imaging of insect retinas and electrode-based measurements of photoreceptors under different light conditions (Frolov & Ignatova, 2020)
        (1) Adult soldier flies were shown to have both low capacitance for reception, as well as a high degree of background noise when measured in dark settings, indicating overall a low light sensitivity (Fig. 6, 8)
    e) Imaging of the entire habitus and developmental steps for the optical morphology (Barros-Cordeiro et al., 2014)
        (1) “The process of pupation (larva/pupa apolysis) occurred in the first six hours, extroversion of the head and thoracic appendages took place between the ninth and 21st hours, and the pharate appeared 21 hours after completing pupation.”

4. **Honey bee (Hymenoptera: Apidae) - *Apis mellifera***
    a) Muscle and flight development in honey bees is less driven by age than by behavior (Roberts & Elekonich, 2005)
        (1) “gene expression in honey bee brains changes relative to behavioral task and help to identify some of the genetic and biochemical mechanisms underlying behavioral transitions in this species”

b) Proteomes (corresponding to behavior and development) in the bee brain differ by role within the hive, with some upregulated for forager vs. nurse bees, etc. (This paper has such a great review of proteome function) (Garcia et al., 2009)
c) Mushroom bodies vary between castes in honey bees (hormone-mediated changes differentiating caste expression) - can be linked to some of the biomarker endocrine review (Barchuk et al., 2018)
    (1) "The brain of a recently hatched honey bee larva is similar to that of other insects and does not seem to exhibit morphological differences between the castes much before the fourth larval instar, L4 (Nelson 1924; Moda et al. 2013)."
    (2) Foraging is also critical to mushroom body growth over time for honey bees, tying also to the plasticity of the honey bee nociception related to environmental exposure (Ismail et al., 2006)
        (a) We report here that foraging bees had a larger volume of mushroom body neuropil than did age-matched bees confined to the hive. This result indicates that direct experience of the world outside the hive causes mushroom body neuropil growth in bees.
d) There have been some classic studies (ie Wigglesworth's landmark book) about how the nervous system develops in holometabolous insects (Edwards, 1970)
    (1) This one reviews the eye and other sensory functions develop
e) The capability of recognizing colony members is a key component of eusociality and rely on some internal references for the perception of nestmates at different life stages (Aquiloni & Tricarico, 2015) < - this

book covers a lot!

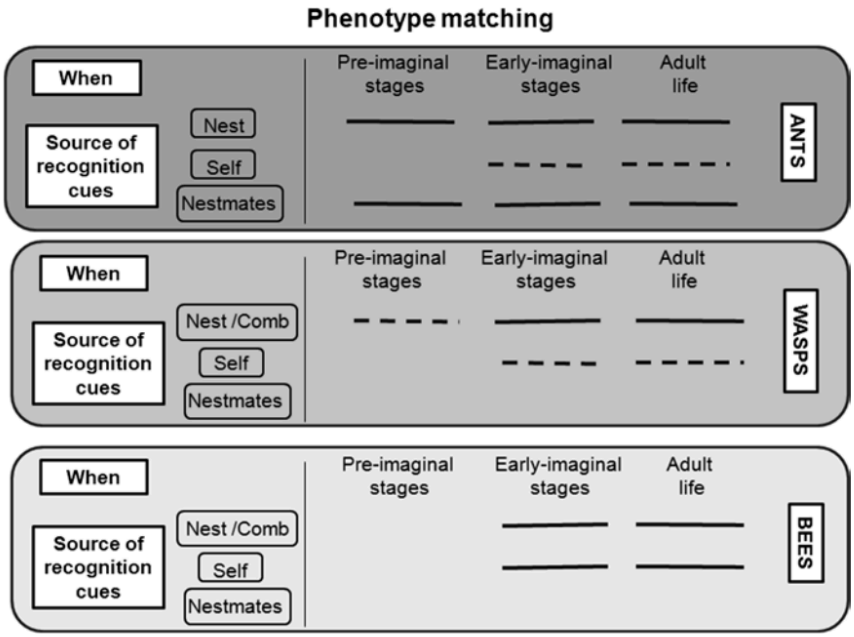


Fig. 10.2 Schematic view of the ontogeny of nestmate recognition ability in ants, wasps and bees according to the phenotype matching model (a possible mechanisms of direct recognition, see text). For each taxonomic group, life stages in which recognition cues can be learned (when) and their source (source of recognition cues) are represented. *Continued lines* indicate factors that have been experimentally shown to play a role in the ontogeny of nestmate recognition, while *dotted lined* indicate factors that are possibly involved

5. **Cricket (Orthoptera: Gryllidae) - *Archeta domesticus* or *Gryllodes sigillatus***
    a) Mushroom body development can be investigated through Kenyon cell labeling, indicating mushroom bodies grow in size from the inside out (Cayre et al., 2000)
    b) Transcriptomes reveal differential expression as the larvae develops, and *A. domesticus* were found to have gregarines contigs within the transcriptome assembly (Oppert et al., 2020)
        (1) Patterns of expression indicated that embryos and 1 d hatchlings often clustered in expression analyses, whereas nymphs and adults usually had similar patterns
    c) Ontogeny of the mushroom bodies of the three most common house crickets (*Gryllus bimaculatus* Deg., *Acheta domesticus* L., and *Schistocerca gregaria* Forsk.) can be linked to homologous gene expression in *Drosophila* (Panov, 2019)
    d) Crickets demonstrate phenotypic, chemosensory self-recognition that is indicative of “online processing” and used when finding mates [picking up on their sensory cues] (Capodeanu-Nägler et al., 2014)
    e) Classic paper on findings related to ganglion development (volume and cell number). (Gymer & Edwards, 1967)

(1) “There are approximately 2,000 association neurons and 100 motor neurons in all stages. The number of glial cells increases from 1,000 to 17,000.”

f) Classic paper on genetic mutations that result in neuronal changes in Polynesian field crickets in adulthood (knockdowns in larvae result in subsequent loss through molts) - this affects the ability to generate synapses from these different neural class types (Bentley, 1975)

6. Silkworm (Lepidoptera: Bombycidae) - *Bombyx mori*

a) The size, slowness of locomotion, and ease of injection have made silkworm larvae excellent model organisms (Sekimizu et al., n.d.)

(1) Table 2

Table 1. Comparision of silkworm with other insects

Items	Silkworm	Drosophila	Honey bee	Waxmoth	Grass hopper
Size	40-60 mm	1-3 mm	15-17 mm	30-40 mm	60-80 mm
Breeding method	Well established (> 5000 years)	Well established	Well established	Established	Established
Locomotion	Larva: slow, Adult: cannot fly	Flies	Flies	Larva: faster than silkworm, Adult: flies	Jumps, flies
Special handling technique	Not required	Required	Required	Not required	Required
Chance of biohazard	Less	Higher	Higher	Higher than silkworm, less than others	Higher
Injection technique	Easier, anyone can learn within couple of hours	Difficult, requires skilled personnel	--	Easier	--
Isolation of organs	Easier	Difficult ,not always possible	Easier	Easier	Easier
Route of administration/ Accuracy of administrated dosage	Oral, injection to dorsal surface: intrahemolymph, intramidgut/ accurate in case of injection	Oral, injection to dorsal surface, not accurate	--	Oral, topical, injection to ventral surface/ accurate in case of injection	--
Diseases models	Many	Many	--	Few	Few

b) Proteome analysis was conducted on the silkworm brain at the 5th larval instar. (Wang et al., 2016)

(1) Characterized protein homologues to *Drosphila.*

c) Odorant binding protein capacity was investigated in the silkworm from egg to adult stages. This is related to sex pheremone perception, as well as transcriptomic expression in multiple tissues of the silkworm body. (Guo et al., 2021)

(1) Male responsiveness to female sex hormones is not age-dependent, but odorant binding protein expression does vary over the life cycle

d) Mouthpart sensilla are critical for search/feeding behavior in silkworms, and research has investigated their ontogeny in the muga silkworm larval-adult development through microscopy. (Goldsmith et al., 2014)

(1) “The types, distribution pattern of the sensilla remained almost the same in the different larval stages except for the number and dimension in some cases”

e) Examination of the relationship of neurosecretory cells in the larva and pupa of *Bombyx mori* has revealed the firing rate at the transition from pupa to adult (related to insulin production). (Suenobu et al., 2004)

f) miRNA (microRNA) genes have been found to be upregulated in the egg and pupal stages, indicating their role within growth and

development, detected as homologues of *Drosophila.* (Zhang et al., 2009)

g) Hox genes have been investigated in *Bombyx mori,* using cloning to knock out genes related to appendage development (*Antennapedia)*. Phenotypic changes of the *wes* mutation of this *Hox* gene were manifested as eyespot malformation in the larva. (Chen et al., 2013)
    (1) There have been a fair number of studies looking at knockdown and gene expression in *Bombyx mori*

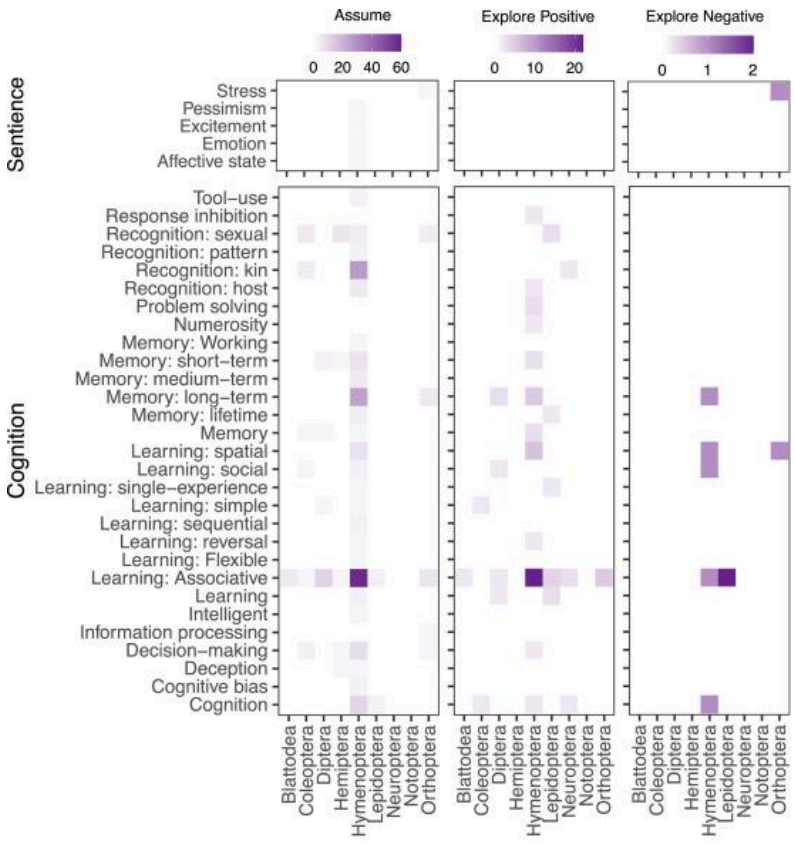
h) Neuropeptides and gene replication/transcription were examined through the transcriptomes of different embryonic stages (Xu et al., 2021)
    (1) “Although pathways of juvenile hormone and the ecdysteroid 20-hydroxyecdysone, and transcription factors were expressed throughout the embryonic development stages, more regulatory pathways were highly expressed around the organogenesis stage, suggesting more gene expression for organogenesis.”

IV. BEHAVIOR/COGNITION/NOCICEPTION

A. Sensory-related behaviors for groups of interest examined by life stage

1. General Insect behavior related to stages

a) Exploration/review of cognitive capacity/behavior of species involved in insect farming (Lambert et al., 2021)



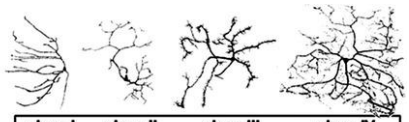
(1)

b) *Manduca* and *Drosophila* have been heavily reviewed for their behavioral responses to painful stimuli. In Manduca, the integration of eggs into the exoskeleton from parasitoids, for example, have been shown to lead to negative behaviors in the Manduca larva. (Walters, 2018)

   (1) *"Manduca larvae, where prominent sensitizing effects of pinch have been found in central neural activity but not primary afferent activity (Tabuena et al., 2017). The neurophysiological alterations in nociceptors and their synaptic targets that have been identified in gastropods and insects involve signaling pathways known to contribute to the induction and maintenance of persistent pain in mammals."* - Walters also examined the defensive behaviors of *Manduca* related to painful/noxious stimuli in the laboratory and field (2001)

c) The responses to noxious stimuli in *Drosophila* larvae and adults have been linked to different genetic assessments that make use of the gene libraries of this model insect (Im & Galko, 2012)

   (1) Classes of larval multidendritic neurons and the nociceptive sensory modalities they mediate.

(2) 

	class I	class II	class III	class IV
Noxious Heat	-	-	-	+++
Mechanical	+	+	-	+++
Chemical	?	?	?	?
Noxious Cold	?	?	?	?

d) Primarily *Drosophila*-informed review of the different classes of neurons (Burrell, 2017)

2. Mealworm (Coleoptera: Tenebrionidae) - *Tenebrio molitor*

a) Adults' numerosity capabilities, tested by chemical ecology (Carazo et al., 2009)

(1) Controlled choice test for adult males to choose between number of female odor sources, where the males were found to choose the choice with the most individual sources

3.* Soldier fly (Diptera: Stratiomyidae) - *Hermetia illucens

a) Larval behavior related to food and compression, with an idea of how group dynamics are induced with population size (Shishkov Dissertation 2020)

(1) High potential for overheating when they are crowded, Shishkov also goes into detail about the standard area recommended/required for rearing. While the larvae can pile on top of each other, they are still limited by physics in terms of how they can withstand crowding

(a) The "fountain of larvae" is not be possible for other animal species. Larvae crawl on top of one another to access food, while cattle and other land animals do not to avoid hurting one another. Fish in schools avoid touching each other, while larvae touch one another constantly, and in fact do not like to be isolated. The feeding behavior of other animals is affected by their social hierarchy, 32,33 while larvae do not have complex social dynamics. Thus, fly larvae are unique among scavengers in their group feeding abilities.

b) Comparison of the oxidative stress of different killing methods for black soldier fly revealed that blanching is the most appropriate/least harm- inducing method to employ (Larouche et al., 2019)

c) Starvation conditions for black soldier fly can reduce the taxonomic diversity of the microbiome, and optimizing the number of symbiotic bacteria that can induce hunger has been hypothesized to be a way to increase production of larval soldier flies (Yang et al., 2021). Starved flies have different biota than that of fed flies.

(1) “Interestingly, their results [about larval cockroach] contrast with those demonstrated in the current study; however, it is hypothesized that such differences could be due to life-history differences with the American roach having a longer life-span (e.g., 12 months) with adults feeding, and the black soldier fly adult have a comparatively short life-span with little reliance on adult nutrition (Rau, 1940; Tomberlin et al., 2002; Bertino-Grimaldi et al., 2013).”

4. **Honey bee (Hymenoptera: Apidae) - *Apis mellifera***
    a) Researchers examined personality and behaviors across time in individuals within an observation hive (little detail on the larval phase behaviors in this study) (Walton & Toth, 2016)
    b) Foraging greatly dictates a lot of the developmental-behavioral complexes in social bees, so access to foraging will greatly influence the life cycle of individuals (Ismail et al., 2006)
    c) Interactions with queen larvae have been used to assess the behaviors exhibited by other adult worker colony members in said interactions, linking it to transcriptome expression within the mushroom body of antagonistic and agonistic individuals (Shpigler et al., 2019)
    d) Interactions between workers and larvae (brood) have shown that the worker is dictating success of the larval development, but the larvae are still responding to stimuli from the worker (Siefert et al., 2021)
    e) Adult honey bees selected between numerical choices

5. **Cricket (Orthoptera: Gryllidae) - *Archeta domesticus* or *Gryllodes sigillatus***
    a) *Archeta domesticus* demonstrate associative olfactive learning ability from sensing necromones and responding with avoidance behavior, in both sexes. (Shephard et al., 2018)
    b) Fasting can cause *Archeta domesticus* to move less, demonstrating less movement/activity throughout an enclosure and less investment in grooming behaviors (Vossen et al., 2022)
    c) Personality traits in *Gryllus campestris* in response to disturbance were measured in individuals in the final instar of the larval stage, finding that individuals exhibiting “bold” behaviors had shorter lifespans (Niemelä et al., 2015)
    d) Family-related responses related to “fear” in *Gryllus integer* were not found in larval individuals, but were found in adults - examination of the “freeze” response in immature and adult crickets that were exposed to a negative stimulus (vibrations from a dropped object) (Hedrick, 2013)
        (1) larvae were reared in captivity from wild-caught females
        (2) Both larvae and adults responded, but the immature crickets had no significant difference in duration of freezing between families

e) Tonic immobility (TI) is considered to be the response that insects have to predators when they freeze and are unable to move (due to muscle and neural reactions). This behavior can be induced in *Gryllus bimaculatus* in response to different sensory and mechanical stimuli in adults, as the individual is sensing the vibrations using the chordotonal organ or sensilla. (Nishino & Sakai, 2021)

(1) TI in the cricket has the following characteristics: (1) TI is induced by physical restraint of the legs, (2) the duration of TI is several seconds to several minutes, (3) during TI, responsiveness to sensory stimulation is decreased, (4) TI is terminated spontaneously or by a mechanical disturbance, (5) catalepsy emerges during TI, (6) TI is used as one of the anti-predator strategies in conjunction with fleeing behavior. Many of these findings for TI in the cricket are similar to those for vertebrates such as birds and mammals (Ratner 1967).

(2) Regarding the mechanisms of TI in the cricket, chordotonal organs which are internal proprioceptors are critically important for inducing TI and the brain is necessary for maintaining immobility of the whole body. In Chap. 8, concrete roles of chordotonal organs in induction and postural control of TI and motor output characterizing TI are described in more detail.

6. **Silkworm (Lepidoptera: Bombycidae) - *Bombyx mori***

a) Stimulation related to feeding behaviors was observed in all larval instars of silkworm in order to identify patterns of locomotion timed with artificial diet sensation and physical stimulation. (Nagata & Nagasawa, 2006)

b) Silkworm larvae were trained and conditioned with an olfactory cue to affect their oviposition behavior (Gámez & León, 2018, 2020)

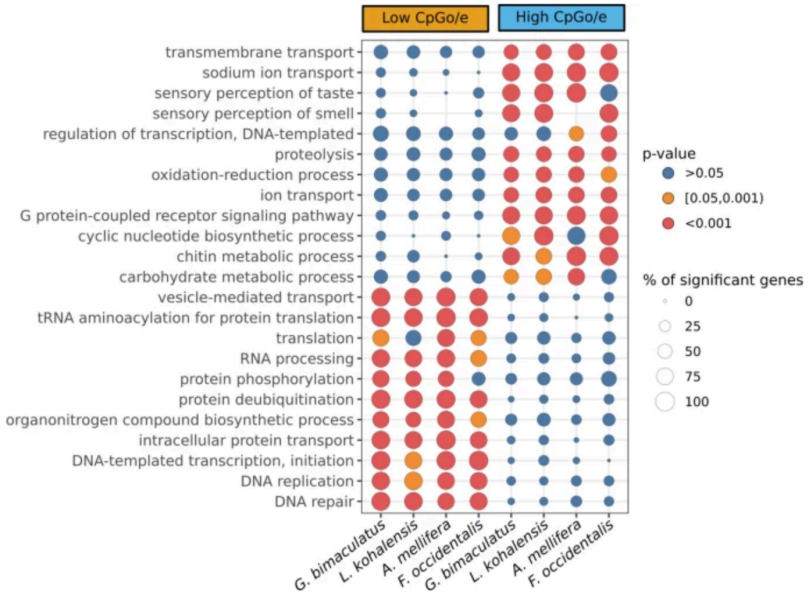
(1) “For this, the larvae learning have to survive the metamorphosis and be shown in oviposition choice. In acquisition phase, a larvae group experienced an odour (conditioned stimulus) paired with mulberry leaves (unconditioned stimulus), another one experienced the odour and the mulberry leaves in an unpaired way and the last one experienced the odour alone during this phase. The results show that when these larvae became moths, only the first group preferred to lay their eggs near the odour when it was present during the test, so that associations learned during the larval stage seem to influence oviposition behaviour during adulthood.”

V. GENETIC INFORMATION TO LINK TO BIOMARKER REVIEW {some more peripheral}

1. **General Insect behavior related to stages**
2. **Mealworm (Coleoptera: Tenebrionidae) - *Tenebrio molitor***

3. **Soldier fly (Diptera: Stratiomyidae) - *Hermetia illucens***
4. **Honey bee (Hymenoptera: Apidae) - *Apis mellifera***
    a) Beekeepers frequently add a super (extra store of honey/pollen) at times when the bees are less able to forage. It's been proposed that beekeepers examine the stress posed to the colony members by the addition of the super through measuring biomarker expression (particularly of the *catalase* and *defensin*). It's been found that the increase in honey flow can cause worker bees to express *catalase* at a higher rate, indicating oxidative stress. (Kadri et al., 2021)
5. **Cricket (Orthoptera: Gryllidae) - *Archeta domesticus* or *Gryllodes sigillatus***
    a) Genome of *Gryllus bimaculatus* reveals that the large size is due to transposable elements, details reasons why the cricket is a good model organism (Ylla et al., 2021)
        (1) Lots of comparison in this paper to annotations relative to the genomes of *Drosophila, Apis, Bombyx*

Fig. 3: Functional differences between high- and low- $CpG_{o/e}$ genes.



    (2)
6. **Silkworm (Lepidoptera: Bombycidae) - *Bombyx mori***
    a) Researchers have been working on genomic profiling in silkworms, establishing biotoxins, vaccines, and proteins within larval or pupal *Bombyx mori* (Kato et al., 2010)
        (1) Great background on the silkworm genome, can be linked to biomarker review
    b) Environmental cues related to heat have been investigated in their genomic expression in *Bombyx mori* (Chen et al., 2020))

VI. CAVEATS

A. The degree of sociality that insects display (for any species that lives in a colony) means that some of the overall functions, individual welfare, and behaviors needed for colony survival can lead to the outsourcing and transfer some of the individual needs to others within the group (Monsó & Osuna-Mascaró, 2020)
    1. The caste of the individual dictates their individual functions within the group overall.
    2. This is a whole other area of social biology - focused on the “superorganism” - something that has been examined for ant and honey bee colonies (Seeley, 1989)
B. Physical trait and genetics limit the development of any cognitive capacity, so if an organism has not developed the morphological/physiological traits needed for cognitive or nociceptive perception, they will not have such perception at a particular life stage
    1. *There is no welfare function if the morphological structure it requires does not exist*

VII. PROPOSED RESEARCH

A. Highlight the gaps in the table and researchers that could potentially fill them based on similar foci/skills in their own programs.
B. Research groups that have studied these taxa or similar questions in the past
    1. Taxa-relevant research groups
    2. Research question-relevant groups

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