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She Shapes Events As They Come: Plasticity in Female Insect Reproduction[1]

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"Reproduction is what bugs do best. It's one of the reasons why they dominate the planet."

–from the IMAX film *Bugs! in 3D* (Slee and Aron 2003)

Abstract

The typical insect ovary has a modular arrangement, with the ovariole as its fundamental modular unit. In general, an increased ovariole number appears to correlate with total potential reproductive output, but other physiological characteristics of the ovary can theoretically influence the rate and timing of egg production as well, the rate of öocyte maturation being one such parameter. Nevertheless, it would be incorrect to imagine that an increased rate of egg production is the only relevant fitness parameter. While insects such as honeybees and drosophilid vinegar ("fruit") flies do seem to be characterized by a maximization of total egg production, there are clearly constraints (or trade-offs) involved even in these examples. The decreased reproductive output potential in worker *versus* queen honeybees, as well as interspecific variation in ovariole number within both of these taxa, suggests that maximization of reproductive output entails some physiological, ontogenetic and/or life history trade-off. More extreme examples are parasitic or viviparous insects (such as tsetse flies) that produce as few as one egg at a time. Furthermore, there is substantial variation across broad and narrow taxonomic groups of insects in the degree to which the rates (öocyte maturation, oviposition) and potential rates (ovariole number) of egg production are phenotypically plastic. Here I will review several well-documented cases of interspecific and/or phenotypically plastic variability in the rates and potential rates of egg production in a wide variety of insect

[1] Title derived from the *Tao te Ching* by Lao-Tzu (chapter 45, S. Mitchell translation 1992).

taxa. I will argue that developing a comprehensive theory of insect reproductive plasticity will require comparative phylogenetic approaches that take account of the interactions between ecological and ontogenetic factors, including developmental constraints. I will close by discussing the apparent similarities between the ecdysis and ovipositional behavioral networks.

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Introduction

It's probably fair to say that the decision of where, when, how much and how often to reproduce is the most important decision (from an evolutionary point of view) that most organisms will make in their lifetimes. But on what basis do they make this critical calculation? What external criteria influence this decision-making process? By what internal mechanisms are these external (environmental) signals integrated to alter development, physiology and/or behavior? And, what features of organisms (morphological, developmental, physiological, historical, etc.) might constrain or bias their responses to these environmental signals? Answering these questions has profound implications for, among other things, evolution, life history theory, conservation biology, and pest control.

Some General Thoughts on Plasticity

The "decision" referred to above is another way of describing plasticity in reproduction, which is generally understood as variation (morphological, physiological, behavioral) within genotypes resulting from environmental heterogeneity (reviewed in Schlichting and Pigliucci 1998; Kalisz and Kramer 2008). Two oft-discussed dichotomies bear consideration: continuous "versus" discontinuous plasticity, and adaptive versus non-adaptive plasticity. An example of continuous plasticity is the effect of rearing temperature on body size, while alternative spring/fall butterfly wing morphology (polyphenism) is an example of discontinuous plasticity. Adaptive plasticity is a plastic response that tends to yield an increase in fitness, such as the effect of day length on the likelihood of entering diapause. Non-adaptive plasticity, by contrast, indicates a plastic response that does not yield a predictable increase in fitness. It is merely, for example, a metabolic reality, such as the general correlation of temperature and developmental rate. Note, that such examples of non-adaptive plasticity are *not necessarily* maladaptive.

There seems to be a general impression [and Schlichting and Pigliucci (1998) as well as West-Eberhard (1989) appear to give this impression] that discontinuous plasticity is often indicative of adaptive plasticity. This seems reasonable for at least two reasons:

1. The discreteness of the morphologies that characterize discontinuous plasticity have obvious alternative fitness advantages in their discrete

environments, some of which have been demonstrated experimentally. Seasonal polyphenisms are famous examples.

2. There are convincing cases where continuously varying traits in basal lineages have been inferred to have become more discrete in derived lineages. The multiple, independent origins of insect eusociality/division of labor is one of the most widely cited examples (see Robinson 1992). Such a progression from continuous to discontinuous plasticity would strongly indicate molding by natural selection.

Less appreciated, though, is the fact that variation in the predictability of environmental signals that induce a discontinuous plastic response can result in any point on a continuum of plastic responses from adaptive to maladaptive in any given instance. For example, consider the use of a temperature threshold as a cue for breaking diapause, as in some ladybug beetles (Hodek 1996). Such a response would be quite maladaptive in the event of an unusually warm day in the middle of winter, since a cold snap the following week could lead to mass mortality. In such a situation, a graded (continuous) response to environmental conditions might be preferable.[2]

An underlying point here is that the adaptive nature of the response can only be judged in an appropriate ecological context. In the laboratory, while the advantage of controlling variables is obvious, the appropriateness of the ecological context can only be approximated. In the field, appropriate ecological contexts can be studied, but it is of course difficult to exclude factors which could not be controlled for (or, even worse, those which covary for trivial and even temporary reasons) as plausible alternative hypotheses to the adaptive hypothesis.

Thus, continuously varying traits may be adaptive in certain contexts, but not in others. Or, rather, the plasticity *per se* may be non-adaptive; for example, it may be a physiological constraint. However, the exact nature of the plasticity (the shape of the reaction norm itself) may be expected to have been tinkered with to provide the most adaptive solution within the context of the physiological constraint(s).

This suggests the hypothesis that most cases of adaptive plasticity may have, at their root, non-adaptive physiological responses which were ultimately molded by natural selection to produce either continuous or

[2] Indeed, such a graded response is found in ladybugs (Coleoptera: Coccinellidae), both within and among species (Hodek 1996), and may be one explanation for the evolutionary success of the group.

discontinuous adaptive plasticity.[3] These ideas are similar to those proposed previously by Schmalhausen (1949) and Matsuda (1987).

Such a situation may be particularly relevant for many instances of insect reproductive plasticity. The general correlation between food intake and ovarian growth is one possible example (see Labeyrie 1978, Wheeler 1996). Since the ovaries can grow in an adult insect in which the majority of structures have stopped growing, this correlation is completely expected, and would hardly be considered an adaptive response. However, modulations in the specific ways in which food induces growth of öocytes are probably good examples of adaptive plasticity. Öocyte growth up to resting stages in the sheep blowfly *Lucilia sericata* is one such example that I consider in some detail below.

Another confusing point is whether the observed continuity is individual or populational. In other words, if different individuals (for whatever reason, be it genetic or epigenetic) have different thresholds or otherwise differ in their environmental sensitivity to the plasticity cues, then a population may be seen to have a continuous response while each individual might have a predictably discontinuous response. Likewise, a discontinuous response may be an adaptive bet-hedging strategy from a populational perspective, while a given individual's response may strike the observer in that specific instance as being maladaptive.

Examples of insect reproductive plasticity cover the spectrum from discontinuous (e.g. soldier *vs.* reproductive castes in termites) to continuous (e.g. the effect of host plant availability on oviposition frequency or specificity, as per Mercander and Scriber, 2005). For the purposes of this chapter, I will focus mainly on instances where the plasticity is or is likely to be adaptive, whether in continuous or in discontinuous traits.

Providing an overview of insect reproductive plasticity that addresses not only the relevant ecological and evolutionary forces, but also the physiological and ontogenetic underpinnings, is a substantial and possibly unwieldy undertaking. From an ecological point of view, there are four major ways in which reproduction can be plastic: number of progeny, timing of reproduction, size/mass/quality of progeny, and place/timing of offspring release. On the other hand, from an ontogenetic point of view, one can think in terms of stages during ontogeny when particular aspects of insect

[3] This is an example of what Gould and Vrba (1982) termed an "exaptation": in this case, a non-adaptive feature of an organism later coopted for its current (adaptive) function. Such a "non-adaptive feature" is what Gould and Lewontin (1979) famously analogized to a cathedral's spandrel.

reproduction are subject to plasticity. In insects, these stages can be roughly divided into ovarian differentiation (the formation of an ovarian primordium from undifferentiated cells), ovarian maturation (also known as öogenesis), and oviposition (see Figure 1). To differing degrees, plasticity in each of these three ontogenetic processes influences the four ecological aspects of reproductive plasticity listed above (summarized at the end of the chapter in Figure 6). For example, I will describe instances in which plasticity in the timing of differentiation influences total number of progeny or the timing of reproductive maturity. Similarly, plasticity in maturation or oviposition can influence the timing, frequency and output of egg laying. As an organizational principle for this chapter, I will adopt an ontogenetic approach, focusing in turn on these three main ontogenetic processes. Within each process, I will attempt to show how plasticity for that process might influence plasticity in the four ecological aspects described above. I will end with a description of other aspects of reproductive plasticity not covered explicitly in the main body of the text.

Overview of Female Insect Reproductive Development

Embryo

In the majority of studied insects,[4] the germ cell primordia exist as groups of distinct, round "pole cells" at the posterior end of the early embryo (reviewed in Büning 1994). Subsequently, the pole cells migrate into the center of the embryo and release signals that recruit the mesodermal cells which will ultimately form the bulk of the ovary (reviewed in Santos and Lehmann 2004). While there has been a large body of research in *Drosophila melanogaster* on the signals and patterning molecules involved in these early events of ovarian development (see Santos and Lehmann 2004), the relationships between early embryonic development and plasticity in adult reproduction has been little explored. Also, different insects differ in their numbers of pole cells (Büning 1994); however, any relationship between pole cell number and adult reproductive plasticity is unknown. Still, it is theoretically possible that pole cell development may be a way in which the adults of some insects "communicate," via maternal effects, the state of the adult environment to their offspring.

[4] Honeybees (genus *Apis*) are a notable exception (reviewed in Büning 1994).

Larva/Nymph

Ovarian tissue proliferation and differentiation usually occurs during the larval/nymphal stage (Buning 1994). Grasshoppers are one exception, where ovarian differentiation is completed in the embryo stage (see Stauffer and Whitman 1997); Lepidoptera are another (see below and Figure 3). Variation in the stage at which differentiation occurs may influence later life-history events (e.g. whether or not the adults feed), as suggested by Büning (1994). I will focus here mainly on the vast majority of insects whose ovaries are subdivided into ovarioles (but see the section on paedogenesis below and Figure 5).

In almost all cases known, the mesodermal cells of the ovary proliferate during the earliest larval/nymph stages, usually without differentiation (reviewed in Büning 1994). In some cases, the germ cells also begin to divide at this time, sometimes forming into germ cell/nurse cell clusters. The stereotyped pattern of ovarian differentiation begins with the formation of ovarioles: öocyte maturation tubes that are the functional unit of the insect ovary (Figure 1). In *Drosophila melanogaster*, the process of ovarian differentiation is entirely regulated by the mesodermal cells of the ovary, as it can proceed in the complete absence of germ cells (Ashburner 1989). Ovariole differentiation in insects (King et al. 1968; Büning 1994) generally begins with the formation, in the anterior of the ovary, of pancake-like stacks of cells known as terminal filaments, which will ultimately cap each ovariole. Posterior to the terminal filament lies the germarium, a mixture of germ cells and mesoderm. These germ cells, when they divide, will produce the öocytes and nutritive nurse cells. Öocytes (with or without nurse cells, depending on the insect's ovariole type) will then be surrounded by follicle cells, forming the incipient "egg chambers." At the posterior end of the ovariole, a stack of cells somewhat analogous to the terminal filament (known as the basal stalk or pedicle) will form in some insects, later connecting to the oviduct (a structure not derived from the ovary anlagen). At this point, ovarian differentiation is complete. In the next phase, öocyte chambers grow into mature öocytes, in a process known as "öogenesis" or "öocyte maturation."

The distinction between ovarian differentiation and öocyte maturation is not merely a semantic one. The maximum number of ovarioles in all insects is fixed during pre-adult stages (coccids are the one confirmed exception; see below). This is significant, because ovariole number is correlated with potential fecundity (David 1970; Cohet and David 1978; Bouletreau-Merle et al. 1982; Stewart et al. 1991), as only one egg at a time can be matured from

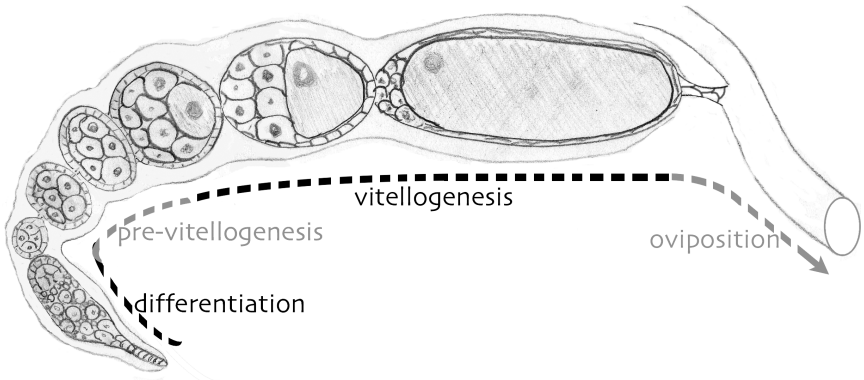

Fig. 1 Schematic drawing of a longitudinal section through an insect ovariole surrounded by its epithelial sheath. Anterior is to the left; the oviduct is the tubular structure at the far right. Ovarian differentiation is the process by which the terminal filaments (the stack of cells at the extreme anterior) form, the initial germ line stem cell divisions take place, and the first egg chambers begin to be surrounded by a monolayer of follicle cells. Note that ovarian differentiation is complete before pre-vitellogenesis begins, while the subsequent stages, in many insects, continue throughout the life of the adult female. Öocyte maturation (or öogenesis) is the growth of the egg chambers, six of which are indicated here. Maturation can be subdivided into a pre-vitellogenic, a vitellogenic, and a choriogenic (not shown) stage (see the text). Mature eggs then pass down the oviduct to be fertilized and oviposited. Öocytes that leave the body are termed eggs. Each of the indicated stages is subject to plasticity, as described in the text.

each ovariole. Hence, a possible determinant of fecundity becomes fixed early in the insect's life, and in some cases as early as the embryo. So although ovariole number is plastic in many insects (see more on this below), this plasticity does not extend into the adult phase. Öocyte maturation, by contrast, can be modulated in many insects during the adult stage (see below). Thus, there are sound functional reasons to consider the two stages of ovarian development—differentiation and maturation—as distinct from one another.

Adult

Insects are highly modular. The imaginal discs in some holometabolous insects are an extreme manifestation of this modularity, where the anlagen (primordia) for different appendages are spatially separated from one another, allowing the different structures the capacity to develop somewhat autonomously (but see Nijhout and Emlen 1998, Nijhout and Davidowitz

this volume). In fact, the phenomenonal variety of insect morphologies can in some sense be accounted for by this modular organization. Still, there is a tremendous constraint on growth in all insects imposed by their hard cuticle and the presence of wings in adults: the transition to the adult stage is the terminal molt (with the single exception of mayflies). Therefore, no alterations in external morphology can be made beyond the adult molt.

The internal, soft structures in insects are not constrained in this way. Fat body can be built up and broken down, as can flight muscles, brain cells, and so on. Ovarian development, too, is extremely mutable during the adult stage. For example, öogenesis shuts down during adult diapause, and then resumes after diapause is broken. Still, as indicated above, there is one important apparent constraint on ovarian development in virtually all insects: maximum ovariole number is fixed during pre-adult stages, since no new ovarioles can be added after that time. This seems curious from a functional point of view. Ovariole number relates in some way to maximal egg production rate (e.g. David 1970; Cohet and David 1978; Bouletreau-Merle et al. 1982; Stewart et al. 1991; see below); thus, it would surely be advantageous for insects to be able to set their ovariole numbers after exploring their adult environment. The clearest example of such an advantage can be seen in insects whose pre-adult and adult habitats are different (as they often are, particularly in the Holometabola and in many aquatic insects), since their pre-adult habitats might well provide no reliable clues as to the state of their adult environment-to-be. The most obvious explanation is that there is some underlying constraint on ovariole differentiation in adults. We will explore this possibility below when discussing the scale insects (Hemiptera: Coccidae), the only insect taxon that is know to be able to substantially increase ovariole numbers as adults.[5]

Six key reproductive features can be modulated in the adult stage: öocyte maturation rate, the number of active ovarioles, egg size, the number of eggs held for oviposition, the timing of oviposition, and the place of oviposition.

Öocyte maturation (which, as mentioned above, often commences in the pre-adult stage; reviewed in Büning 1994) begins with the separation of egg

[5] A second group of insects—termites—are often cited as having this capacity as well. Many references are made to a study by Truckenbrodt and Amelung (1986) on *Odontotermes stercorivorus* (Termitidae) queens, showing that they increase their ovariole numbers by fission of pre-existing ovarioles. However, these authors only reported on ovarian growth in queen nymphs through the 5th stadium. Thus, although I consider it highly likely that this species can also add ovarioles as adults, this has not to my knowledge been technically shown.

chambers from the germarium (Figure 1). These egg chambers will then grow in size, taking up structural materials from the nurse cells and/or through metabolic processes in the öocytes themselves. This first stage of maturation is referred to as pre-vitellogenesis. In vitellogenesis (the 2nd stage), the öocyte begins to incorporate yolk proteins either from the hemolymph or, less often, from proteins produced in the follicle cells. This stage is typified by rapid öocyte growth. During choriogenesis (the 3rd stage of maturation), the chorion is deposited. The mature öocyte is then ovulated into the oviducts, fertilized, and oviposited (laid). The scenario outlined above is true for most insects, although there are exceptions, such as in parasitic and viviparous forms, where embryogenesis can precede the onset of vitellogenesis, and choriogenesis can be skipped entirely (reviewed in Wheeler 1996; Grbic 2003).

Generally, each mature ovariole contains a linear series of developing egg chambers, resulting in an anterior-posterior progression from germ cells, to incipient egg chambers, to mature öocytes (Figure 1). In many insects, the primary (posterior-most) öocyte in each ovariole matures in synchrony, so that each ovariole contributes one egg during each reproductive (gonotrophic) cycle. Other patterns include those species in which multiple öocytes within each ovariole undergo vitellogensis and mature simultaneously, while other species can have highly asynchronous öocyte maturation across ovarioles. Some insects oviposit batches of eggs into a single clutch, while others lay eggs singly. These features vary widely among insects, and are often plastic.

Öocyte maturation is famously plastic, and can be affected by food availability, the presence of males, oviposition site ("host") availability, temperature, humidity, day length, pathogens, parasitoids, and so on (reviewed in Labeyrie 1978, Wheeler 1996, Hopkins and Ekbom 1999, Tammaru and Javois 2000, Papaj 2000). Such plasticity will be a major topic of discussion below. Females can hold many mature öocytes awaiting an appropriate oviposition site. Developing öocytes, and even sometimes mature, unlaid eggs can also be resorbed, and the resources therein reallocated for other energetic needs or for future reproduction (Sundburg et al. 2001, Osawa 2005; reviewed in Bell and Bohm 1975). The numbers of mature eggs being held by the female is often referred to as "egg load," and has become an important characteristic in distinguishing reproductive patterns among insects (see Papaj 2000; Jervis and Ferns 2004, 2005).

Oviposition (the passage of eggs or embryos from the body to the environment) is itself subject to plasticity, as insects can judge the relative

appropriateness of sites for the protection and/or growth of their offspring. The degree of clutch size and oviposition site plasticity, and the cues that elicit such plasticity can vary among species and populations (e.g. Fordyce 2005; Haribal and Renwick 2005; Mercander and Scriber 2005). Oviposition can also be modulated by many of the factors cited above that influence öocyte maturation (see above references and Hinton 1981), and facultative viviparity can lead to a decision to either oviposit or brood internally (see Schal et al. 1997). Finally, oviposition in many iteroparous (sequentially ovipositing) insects has been shown to modulate öocyte maturation, as well (reviewed in Papaj 2000).

I will discuss plasticity in a variety of insect groups in ovariole number, öocyte maturation rate (including reproductive diapause), clutch size, egg size, oviposition timing, and place of oviposition . I will consider how plasticity in these processes differ in solitary versus social insects, long-lived versus short-lived adults, r versus k selected species, and parasitic versus free living forms. I will then review some instances of plasticity in overall reproductive mode (viviparity, larval reproduction), and conclude by advocating a broad-based comparative strategy designed to integrate mechanistic (hormonal, genetic, cellular, biochemical), ecological and evolutionary approaches, to reach a holistic understanding of the startling variation in insect reproductive patterns.

Setting the Stage: Plasticity in Ovariole Number

Insect species vary widely in number of ovarioles per ovary, from one to several thousand. Likewise, individuals within populations vary in number of ovarioles, and in some cases this important reproductive characteristic has been shown to be plastic. Because all ovarioles can theoretically mature eggs simultaneously, maximum potential reproductive output correlates positively with ovariole number (David 1970; Cohet and David 1978; Bouletreau-Merle et al. 1982; Stewart et al. 1991; plus many examples discussed below). However, large ovaries can generate problems for lift and flight (Berrigan 1991), and also, rates of öogenesis may be inversely related to number of ovarioles and developing öocytes. These and other trade-offs suggest that ovariole number might be shaped by natural selection. Furthermore, differences in optimal ovariole numbers might be characteristic of different environmental conditions (see below), and selection should favor plasticity for this character in insect populations existing in fluctuating environments.

Several aspects of the pre-adult environment can influence the numbers of ovarioles in the adult, including temperature, food quality, food abundance and crowding (e.g. Saviliev 1928, Robertson 1957, Hinton 1981, Rhamhalinghan 1986; Grenier and Nardon 1994; Delpuech et al. 1995; Morin et al. 1997; Moreteau et al. 1997, Hodin and Riddiford 2000a, Tu and Tatar 2003). In general, higher quality, abundant food assimilated in uncrowded conditions leads to an increase in the ovariole number. The temperature effect on ovariole number, by contrast, is a bell-shaped function, with a certain moderate temperature (which varies widely among populations and species) leading to a maximal ovariole number (see below).

It is tempting to consider the effects of pre-adult feeding on ovariole number to be adaptive and anticipatory, whereby females use current conditions to predict future conditions. It would follow, for example, that during a "poor" reproductive season, it may be advantageous for a females to reduce her number of ovarioles, and instead direct more resources into simply staying alive. Still, a purely correlative explanation for such a pattern cannot be excluded. For example, ovariole number clearly correlates with body size (e.g. Stewart et al. 1991; Gasser et al 2000, Tu and Tatar 2003; reviewed in Honek 1993), so ovariole number differences resulting from differential feeding *per se* are by no means indicative of adaptive plasticity. As I stressed above, phenotypic plasticity does not have to be adaptive, nor to have undergone selection for the plastic response.

Likewise, temperature effects on ovariole number might also be, in essence, a non-adaptive bio-physical plastic response, with the optimum temperature merely representing the metabolic optimum for the molecules involved in terminal filament formation. Still, comparative studies among drosophilid vinegar ("fruit") flies (Diptera: Drosophilidae) demonstrate predictable geographic differences in temperature optima for ovariole number (e.g. Delpuech et al. 1995; Moreteau et al. 1997, Morin et al. 1997, Karan et al 1999, 2000; Gibert et al. 2004; Wayne et al. 2005). Thus, the bell shape of the reaction norm might be a purely physiological reality, whereas the particular nature of the reaction norm (e.g. the optimum temperature and the steepness of the curve; e.g. Gibert et al. 2004) may shift under different selection conditions. In this way, non-adaptive plasticity might give way to an exaptation (see footnote 3, above) allowing for adaptive evolutionary shifts in the mean numbers of ovarioles in different populations or species.

One way of testing this hypothesis is to compare the mechanisms underlying the plastic response with those underlying genetically-fixed differences among related populations or species. We (Hodin and Riddiford

2000a) undertook such a test, comparing food and temperature-induced plasticity to intra- and inter-specific variation among members of the *melanogaster* species group of *Drosophila* (Figure 2). We reasoned that since maximal ovariole number is set before metamorphosis in vinegar flies, any differences in ovariole number between two flies must be either due to ontogenetic differences in the processes of ovarian differentiation in larval stages, or to subsequent cell death and removal of differentiated ovarioles. We used this reasoning to compare trajectories of ovarian development within and across species, as well as in flies raised under a variety of temperature and food conditions. We showed that the ontogenetic mechanisms underlying within- and across-species variation in ovariole number were broader than the mechanistic underpinnings of the plastic responses in *D. melanogaster* (Table 1). In other words, only a subset of the mechanisms underling genetically-based differences (among populations and species) demonstrated plasticity under a variety of food and temperature conditions.

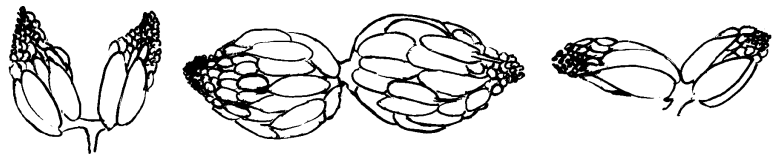

Fig. 2 Gross similarity in ovarian development in *D. melanogaster* when reared in food-limiting conditions, and its sister species *D. sechellia* when fed *ad libitum*. See Hodin and Riddiford (2000a) for details.

At first glance, these results seem to refute the exaptation hypothesis outlined above. However, our plasticity experiments with *D. melanogaster* larvae demonstrate that ovarian growth and ovariole differentiation are processes that can be decoupled under a variety of environmental conditions. The fact that these processes are not *necessarily* correlated suggests the possibility that they can be independently acted upon by natural selection, ultimately yielding the variety of mechanisms underlying ovariole number differences across populations and species noted above. Hence, the exaptation scenario might indeed be valid. Further tests with a diversity of insect groups would help resolve this situation.

For example, comparative studies on Hawai'ian drosophilids (a subfamily separated by at least 60 million years from the branch containing the

Table 1 The array of mechanisms underlying within- and across-species differences in ovariole number in the *melanogaster* species group of the genus *Drosophila* are more broad than the mechanisms underlying plasticity for ovariole number in *Drosophila melanogaster*. Control conditions were the same in all cases : 25°C rearing temperature, uncrowded conditions, full amounts of food. Food reductions were half rations. Mechanisms: **a**=smaller ovarian primordium, 2nd larval stage ("instar"); **b**=slower ovarian growth, 2nd instar; **c**=slower ovarian growth, 3rd instar; **d**=delayed onset of terminal filament (TF) formation; **e**=reduced rate of TF formation. Note: none of the observed differences in ovariole number was due to increased rates in larval ovarian cell death, reduction of ovariole number in the pupal or adult stage or early completion of TF formation. Data compiled from Hodin and Riddiford (2000a).

species	*population / locality*	*rearing condition*	*mean ovariole number*	*mechanism(s) underlying ovariole number difference (all relative to Sevelen control unless indicated)*
melanogaster	Sevelen / Sevelen, Switzerland	control	21.1	
melanogaster	Sevelen / Sevelen, Switzerland	low temp. (15°C)	11.8	**d** and/or **e**
melanogaster	Sevelen / Sevelen, Switzerland	high temp. (30°C)	17.7	**d** and/or **e**
melanogaster	Sevelen / Sevelen, Switzerland	food reduction	18.2	**e**
melanogaster	Capitol Hill / Seattle, USA	control	21.3	no significant difference
melanogaster	Capitol Hill / Seattle, USA	food reduction	18.9	**d** and/or **e**
melanogaster	Nahal / "Evolution Canyon", Israel	control	18.6	**c**, **d**
simulans	"hond" / Zamorano, Honduras	control	18.9	**d**
simulans	"st" / Florida City, USA	control	15.5	**b**, **e?** (relative to Sevelen and *simulans* Honduras)
mauritiana	Riviere Noire, Mauritius	control	12.9	**b**, **d**
sechellia	Cousin Island, Seychelles Islands	control	8.5	**a**, **c**, **d**, **e**
yakuba	Cote d'Ivoire	control	12.9	**a?**, **b**, **c**, **d**, **e**

melanogaster species group) have revealed an extreme range of mean ovariole numbers in different species (from 1 to 50 per ovary; Kambysellis and Heed 1971). Furthermore, this ovariole number variation correlates with profound

ecological differences among the various species. For example, species that oviposit many eggs at once under bark have high ovariole numbers, whereas those that oviposit only one egg at a time on decaying leaves are characterized by lower ovariole numbers. An adaptive explanation for the former seems obvious: higher potential öocyte maturation rate. An explanation for the latter might be found in experiments suggesting that ovary weight is negatively correlated with lift production in a flesh fly (Berrigan 1991), and that wing to thorax ratio (but, importantly, not body size) shows a significant positive correlation with ovariole number across species of the *obscura* group (Drosophilidae; Moreteau et al. 2003). Furthermore, these differences in ovariole number/oviposition strategy correlate with phylogeny (Kambysellis et al. 1995), as there is a general progression among Hawai'ian drosophilids from ancestral decaying leaf laying specialists (1–4 ovarioles per ovary), to decaying stem-laying specialists (5–11 ovarioles per ovary), to highly derived, decaying bark laying generalists (12–50 ovarioles per ovary).[6] In any case, this range of ovariole numbers among Hawai'ian species would provide an independent case with which to explore the mechanistic underpinnings of ovariole number plasticity and variation, and to test the exaptation scenario introduced above, in a phylogenetic context. Plasticity for ovariole number has not been examined for any Hawai'ian species of which I am aware.

Whereas the Drosophilidae might contain the best documented examples of ovariole number disparity (Pappas and Engstrom 1974; Mahowald and Kambysellis 1980), ovariole number variability is found in almost every insect group that has been examined. In Table 2, I organized ranges of reported ovariole numbers (previously compiled by Büning 1994 and Robertson 1961) according to insect order (I have excluded the social insects, which I consider separately below). The first trend that is obvious is that many groups of insects show substantial interspecific variation in ovariole number, notably the Diptera, Neuroptera and Orthoptera. Interestingly, the Lepidoptera and most Hemiptera show surprisingly little variation in ovariole number. The Lepidoptera represent a striking case, in which all nine genera examined have the same number of ovarioles (4). This is particularly intriguing given the vast differences among butterflies and moths in body size, and their ecological and reproductive patterns (reviewed in Ramaswamy et al. 1997), from those that do not feed as adults

[6] While this pattern (from specialist ancestors to generalist descendants) might seem counterintuitive, Van Valen (1965) accounted for such a pattern of expanded "niche width" in derived, island populations.

Table 2 Variation in ovariole numbers in six different orders of insects. The Coleoptera data are from Robertson (1961), and the Orthoptera data from Stauffer and Whitman (1997). All other data are from Büning (1994), with four extra lepidopteran species added [the hawk moth *Manduca sexta* (Nijhout and Riddiford, 1974), the monarch butterfly *Danaus plexippus* (Urquhart 1960), the apple coddling moth *Cydia pomonella* (Benz 1969), and the yucca moths *Tegetigula* spp. (Nielsen and Kristensen 1989)] in order to further validate the striking pattern among the Lepidoptera. I have only included those orders for which Büning (1994) listed data for genera from at least 3 different families. In the Hemiptera, the coccids and the psyllids may be special cases, as described in the text, and were thus excluded. *Meloe*, which is reported to have approximately 1000 ovarioles per ovary, is such an extreme outlier for the Coleoptera that it was excluded here (see the text). I treat the social insects and their close relatives (the Hymenoptera and the Dictyoptera + Isoptera) separately (see below). Clearly, there are many genera with reported ovariole numbers that were not included in Büning's review, so this list should not by any means be considered comprehensive. I merely intend to demonstrate the broad, inter-ordinal trends here.

order	*number of genera examined*	*number of families represented*	*range of ovariole numbers per ovary*
Orthoptera	33	7	2–150
Hemiptera (except coccids and psyllids)	15	11	4–15
Coleoptera (except *Meloe*)	223	31	1–70
Neuroptera	4	4	10–160
Lepidoptera	9	9	4
Diptera	10	8	1–150

and eclose with their full complement of mature eggs (such as the silk moth *Bombyx mori*, where only oviposition is plastic in adults), to those with an extended adult phase that eclose with their ovaries in a completely pre-vitellogenic state (including the monarch butterfly *Danaus plexippus*, where all reproductive plasticity is manifest in the adult stage).

Within orders, we can tentatively distinguish a few consistent patterns. For example, in the Diptera, ovariole numbers are lowest in specialist taxa that brood their offspring (such as the tsetse fly), and highest in generalists that exploit rich, plentiful and ephemeral food resources (such as decaying fruit). Beetles that oviposit on dependable resources of borderline nutritional value (k-selected taxa, such as the flour beetle *Tribolium castaneum*) have few ovarioles (4 ovarioles per ovary), while those that feed on episodic, high

nutrition resources (r-selected taxa, such as aphid-feeding ladybugs in the genus *Coccinella*) have many more ovarioles (from 10–60 or more ovarioles per ovary). Non-nesting dung beetles (Coleoptera: Scarabeidae) of the sub-family Aphodiinae have multiple ovarioles per ovary (seven, for example, in *Aphodius fossor*), while advanced nesting dung beetles of the sub-family Scarabeinae all have only one ovary which has exactly one ovariole (Halffter and Edmonds 1982). Still, I would caution the reader from making any firm conclusions based upon these results. With the exception of very few studies, such as that previously described for the Hawai'ian drosophilids, such ovariole number comparisons have not been subjected to rigorous phylogenetic analyses. It is critical that such a strict comparative analysis be done, on a broad assemblage of insect taxa, before we can hope to paint a complete picture of the relationship between reproductive ecology and ovariole number. As I will argue later, such analyses would be one way to identify the constraints (be it developmental, physiological or phylogenetic) that so clearly interact with insect ecology to mold the evolution of insect reproduction.

What are we to make of the non-conformist taxa indicated in Table 2? I argue that the explanations are both ecological and ontogenetic. Take the case of the blister beetles (family Meloidae) from the genus *Meloe*. This fascinating group of insects is perhaps best known for their hypermetamorphoses, in which several distinct larval morphologies are produced in turn, each one specialized for egg predation, bee parasitization, mimicry, overwintering and so on (reviewed in Gillott 1995). Less well known is their massive reproductive potential: *Meloe proscarabaeus* is reported to have approximately 1000 ovarioles per ovary (Büning 1994). This high reproductive potential is translated into enormous bouts of egg laying, in which the beetles dig a hole and therein deposit their immense pile of eggs (as many as 4218 eggs oviposited in one location in *M. cicatricosus*; Fabre 1857). How do these females handle such a tremendous egg load? Apparently, the gravid adult females, which had fattened themselves within the colonies of mason bees (genus *Anthophora*), simply drop to the ground and find an appropriate oviposition location by walking (Fabre 1857). The mostly sedentary lifestyle of these adults is associated with their greatly reduced wings. Flightlessness and enlarged abdomens (physogastry) are seen in other meloid species, sugesting that these insects trade-off wings for ovary development, a topic that we will return to below. Thus, *Meloe* females may have relieved themselves of the trade off between ovariole number/ovary size and foraging/dispersal function by largely avoiding the need to expend energy in either foraging or dispersal.

Interestingly, there is substantial variation in egg production within the family Meloidae, and this variation appears to be associated with proximity of hosts for the larvae. In those meloids, for example, that attack locust egg pods, females deposit their clutches in the general vicinity of such egg pods. The total numbers of eggs deposited by these meloids is 10 or more times lower than in those bee parasitic meloids, whose larvae wait in flowers for a bee visitation so that they can hitch a ride back to the bee nest (Hinton, 1981). Since *M. cicatricosus* larvae must encounter and attach to a mason bee, each larva presumably has a fairly low probability of survival to reproduction (Robertson 1961). Hence, females should produce many larvae. Recently, cooperative behavior has been described for clutches of synchronously-hatching *M. franciscanus* larvae, who can mimic the female pheromone of their mason bee hosts, *Habropoda pallida* (Saul-Gershenz and Millar 2006). The pheromone attracts males, which the groups of larvae respond to by quickly arranging themselves in such a way as to increase the chance that one (or usually many) of their number will be able to attach to the male bee. These lucky larvae then transfer to the female bee during mating attempts, and finally get transported to that females nest to feed on pollen, nectar and eggs. In sum, the particular level of reproductive output of meloid beetles is consistent with life history theory, which predicts, all other factors being equal, a negative correlation between chance of survival to adulthood and egg number (Stearns 1992). Hinton further provides evidence that such egg production strategies correlate more with ecology than taxonomy. A rigorous phylogenetic approach would assist greatly in deciding this issue.

A second non-conformist group is the scale insects (Hemiptera: Coccidae). I excluded this group from Table 2 as they are the only insects[7] known to add substantial numbers of ovarioles during the adult stage (Weglarska 1961, Büning 1994). As indicated previously, this ability would seem to be highly advantageous to any insect whose adult habitat differs from their pre-adult habitat, since they would then be able to optimize ovariole number after assessing the quality of their adult environment. Long lived adults, which may be expected to experience temporal shifts in their adult environment during the adult period, would also be predicted to gain finer control towards reproductive optimization if they had the ability to add ovarioles after adult eclosion. Ovarian development in the ovoviviparous scale insect *Quadraspidiotus ostraeformis* has been studied in the most detail, and ovarioles are added by budding off ovarioles from undifferentiated cells in their tubular gonad (Weglarska 1961). In fact, this process is so divergent

[7] see footnote 5, above

from the mode of ovariole differentiation in typical insects that Weglarska suggests that *Q. ostraeformis* has no ovarioles *per sé*. However, since the follicles do develop along progressions of stages in these buds, they are certainly similar to ovarioles, and for clarity I will continue to use that term here. The epithelial sheath degenerates as ovarioles form in these coccids, which is presumably one of the reasons why the continual process of ovariole differentiation is mechanistically possible in the group. The permanently ensheathed ovarioles of typical insects (see Figure 1), and their apparent lack of pluripotent ovarian cells (see Kirilly and Xie 2007), would seem to explain why they do not retain the ability to add ovarioles as adults (via some sort of constraint). The nature of this constraint presumably has to do with the process of oviposition. The sheaths in typical insect ovaries separates the ovarioles from one another and from the body cavity, and the ovarioles are connected to the lateral oviduct in order to conduct the eggs there in preparation for ovipostion (see Figure 1). By contrast, adult *Q. ostraeformis* females have no such sheaths surrounding their individual ovarioles. Instead, they only have thin peritoneum surrounding the entire ovary, within which they brood their young (Weglarska 1961). As a result, the ovarioles can project any which way into this peritoneal cavity. Such a release from the constraints inherent in typical ovarian morphology would, thus, have resulted in a highly modified process of ovarian morphogenesis.

An analogous situation is found in the paedogenetic (larvally-reproductive) gall midges, which truly lack ovarioles altogether (see Figure 5 and below for more on this group). Indeed, even non-paedogenetic gall midge species lack true ovarioles, as their egg tubes form by secondary fusion (Matuszewski 1968), rather than the typical assembly-line process described in the introduction and illustrated in Figure 1. This derived pattern of ovarian morphogenesis in the non-paedogenetic gall midges may have been one of the features that preadapted[8] that taxon for the evolution of paedogenesis, something which occurred at least twice independently in the group (Hodin and Riddiford 2000b).

The third non-conformist group is the jumping plant lice (Hemiptera: Psyllidae), which have been reported to contain up to 100 ovarioles per ovary (Büning 1994), 10 times the number found in typical hemipterans (see Table 2). Many psyllids are major crop pests, including the Asian citrus psyllid *Diaphorina citri*. This species can survive for months as adults awaiting appropriate oviposition conditions: young, furled leaves. When

[8] *sensu* Gould (1984): features adapted for one function, that are fortuitously suited for another.

such conditions arise, the insects can utilize their high reproductive potential to lay as many as 800 eggs in a few days (Mead 2002), an ability undoubtedly enhanced by the high ovariole numbers characteristic of psyllids. The fact that the adults can feed on mature leaves, while their offspring require young leaves, has two important consequences: 1) it allows the adults to mature eggs while awaiting the appearance of the young leaves for their offspring; 2) it allows adults with high egg loads to remain on their host plant, reducing the need to disperse to find an oviposition site. As in the case of *Meloe* discussed above, such a situation obviates the typical trade off between ovariole number/ovary size and foraging/dispersal function by largely avoiding the need to expend energy in foraging and dispersal. Again, the explanation for their deviant numbers of ovarioles appears to be both ecological and ontogenetic.

The observation that many of the aforementioned trends in ovariole number variation apply across different insect orders makes the situation in the Lepidoptera all the more striking. How can we account for the apparent total lack of variation in ovariole numbers in the Lepidoptera? The patterns that we have described above, where different ecological parameters correlate with differences in ovariole number across several insect orders, indicate that ovariole numbers are subject to natural selection. However, these same selective criteria would presumably apply to Lepidoptera as well. The nine families of Lepidoptera noted in Table 2 include species with a broad range of ecologies and life histories, from r to k strategists, unitvoltine to multivoltine, generalists to specialists, migratory to non-migratory species, short lived to long lived adults, tropical to temperate forms, small to large body size, and so on. Also, they include representatives from at least two relatively basal lepidopteran groups, the leafroller moths (*C. pomonella* from the family Tortricidae) and the yucca moths (family Prodoxidae), in addition to several highly derived families (Kristensen and Skalski 1999). Still, all have exactly 4 ovarioles per ovary.

Such a situation represents a perfect candidate for a developmental constraint (see Hodin 2000). But what is the nature of this constraint? Very little is known concerning the ontogenetic processes underlying ovariole differentiation in the Lepidoptera (Büning 1994). Interestingly, ovariole number determination in female Lepidoptera takes place in the embryo, and is coincident with sperm follicle tube formation in males; male embryos also form four gonadal tubes per gonad (Figure 3; Grünberg 1903). By contrast, testis and ovarian differentiation follow quite distinct ontogenetic routes in most non-lepidopteran insect species (reviewed in Büning 1994), again suggesting an additional and unique level of constraint on lepidopteran

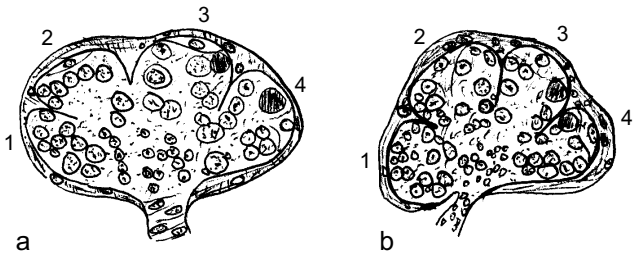

Fig. 3 Cross sections through the embryonic gonads in *Bombyx mori*, redrawn from Grünberg (1903, Figures 1 and 18). (a) embryonic ovary; (b) embryonic testis. The four ovarioles (numbered in a) are already evident at this stage (see also Beckemeyer and Shirk 2004), much earlier than is the case in most insects (reviewed in Büning 1994). Homologous gonadal tubes are differentiating in the testis as well at this stage (numbered in b). In typical insects, ovarian differentiation follows a quite distinct ontogenetic trajectory from testis differentiation (reviewed in Büning 1994). This early ovariole differentiation and the similar male and female ontogenetic trajectories in these moths may help to explain the apparent total lack of variation in ovariole number across the Lepidoptera.

gonadal development. In other words, it is possible that the tightly coupled (canalized?) processes of male and female gonadal formation in Lepidoptera have constrained their evolutionary potential.

One useful avenue for investigating the nature of such (presumed) constraints is to examine the analogous processes in an unconstrained outgroup. The caddis flies (order Trichoptera) are widely accepted as the sister group to the Lepidoptera (e.g. Kristensen 1984; Wheeler et al. 2001). Since the net-spinning caddis fly *Parasthenopsyche sauteri* has approximately 130 ovarioles per ovary (Matsuzaki 1972), caddis flies do not appear to be similarly constrained. Comparative studies on ovarian differentiation in caddis flies and lepidopterans might, therefore, yield some insight into the presumed constraints on ovariole determination in lepidopterans. Despite the wide geographic distribution, ease of collection and high population densities of larval caddis flies, ovarian differentiation remains mostly unstudied in the group (Büning 1994), so comparative data are lacking.

Dividing Up Who Goes into Labor: Ovariole Number Plasticity in Social Insects

By far the most extreme examples of ovariole number plasticity are the queen-worker differences in ovariole numbers in many social insects (Keller 1993). Although data in some taxa suggest a genetic component to queen-worker determination (Michener 1974), by far the predominant mechanism involves differential feeding of larvae (reviewed in Wheeler 1986). So, when

a female honeybee larva is born, she has the potential of developing either as a queen or a worker, depending upon the quantity and quality of food that she receives during larval development (Beetsma 1979). If she gets the queen ration, then she ecloses with up to 200 ovarioles in each ovary, and a fully functional reproductive system with sperm storage organs and reproductive fluid ducts. If, by contrast, this larva only receives the meager worker food allotment, then she ecloses with less than 10 ovarioles per ovary, an underdeveloped sperm storage organ, and an inability to mate (Michener 1974).

The multiple independent origins of sociality within the Hymenoptera, and the entirely independent origin of sociality in the termites (order Isoptera), provide fertile ground for testing evolutionary hypotheses regarding, for example, the mechanisms underlying plastic and genetically-fixed differences in ovariole numbers. Caste differences in ovariole numbers between queens and workers have evolved independently in many social taxa (Keller 1993). This finding, in and of itself, indicates two important points: first, that there is strong selection pressure for increases in ovariole number to maximize reproductive output (in queens/reproductives); and second, that these high ovariole numbers involve trade-offs with non-reproductive tasks (in workers vs. queens/reproductives). Thus, the often lower ovariole numbers found in reproductive females from non-social species when compared to their social counterparts indicates that the former are trading-off potential reproductive output against non-reproductive functions, such as flight, foraging, defense, somatic growth and so on. The extreme specialization of queens for reproduction in many social species releases them from such trade-offs.

An examination of the situation in queenless social insects provides some potent support for the presence and importance of such trade-offs in social colonies, though in this case with respect to öocyte maturation rather than ovariole number. For example, the queenless ponerine ant *Pachycondyla (=Ophthalmopone) berthoudi* has variable proportions of reproductives (gamergates), depending on colony conditions (i.e. they are polygynous). Sledge et al. (1999) compared several such colonies, examining the behavioral profiles of individual ants, and then dissecting them to examine their state of öocyte maturation. The gamergates in colonies with high proportions of reproductives were much like workers in their colony task profiles, and had only moderately mature ovaries. By contrast, gamergates in colonies with a low proportion of reproductives performed no colony labor tasks, and had many more mature ovaries. Ito and colleagues (1996) obtained similar results for the East Asian ponerine ant *Odontomachus*

rixosus, another species with scores of gamergates in each colony. In *Ectatomma tuberculatum*, a Central and South American ponerine species with facultative polygyny (Cook 1905), there is a strong, age-independent correlation between colony duties and reproduction: nurse ants had more mature ovaries and laid trophic eggs (to be fed to larvae), while forager ants had degenerate ovaries and well-developed poison glands (Féneron et al. 1996). Thus, the plasticity of queenless/polygynous ants for reproductive versus worker tasks is functionally related to ovarian developmental plasticity. An examination of the hormonal mechanisms underlying such a trade-off would be extremely edifying.

One widespread misconception concerning reproduction in social insects is that workers are "sterile." This is only technically true in a handful of highly derived social insects, such as the stingless bee (Apidae: Meliponini) species *Frieseomelitta varia* and *Trigona minangkabau* (Cunha et al. 1986; Suka and Inoue 1993), the Ceylon Black Termite (Isoptera: Termitidae) *Hospitalitermes (=Eutermes) monoceros* (Bugnion 1909) and the fire ant (Hymenoptera: Formicidae) *Solenopsis invicta* (Hölldobler and Wilson 1990). In the vast majority of social insects, workers have functional ovaries, but full öocyte maturation and oviposition is repressed (to varying degrees in different taxa) by the presence of a queen or queens (e.g. Michener 1974, Hölldobler and Wilson 1990, Noirot 1990). Thus insect sociality is not the just-so story of queens who have all of the offspring, and workers who toil away their whole lives for the sole inclusive fitness advantage inherent in the reproductive potentials of their little sisters and brothers. It is true that workers in many social hymenopteran taxa are incapable of mating (though there are some notable exceptions that I will discuss below), but they still retain the potential of laying unfertilized (male-producing) eggs that can develop and ultimately mate with conspecific queens. The varying degrees to which reproduction in such workers is held in check by the presence of the queen is a topic that I will now consider.

Let's begin by reviewing the reproductive potential of workers and queens/reproductives in two different social taxa: basal termites and honeybees. Later I will return to these same taxa, and also include a discussion of the stingless bees and the multi-queen (polygynous) ants. If the reader's favorite social insect is not among these four groups, he or she can find information on other social taxa in one of several excellent reviews (Wheeler 1986; Engels and Imperatriz-Fonseca 1990; Peeters 1991; Keller 1993; Peeters 1993; West-Eberhard 1996; Robinson and Vargo 1997; Thorne 1997; O'Donnell 1998; Reeve and Keller 2001; Thorne and Traniello 2003; Hartfelder and Emlen 2004; Schwarz et al. 2007).

Isoptera

All termites are social, and are thought to have arisen from a cockroach-like ancestor. Phylogenetic data, as well as the incipient sociality in some cockroach taxa, provides strong evidence for this evolutionary scenario (reviewed in Thorne and Traniello 2003). Two key features distinguish sociality in the Isoptera and the Hymenoptera. First, termites are hemimetabolous insects, whose pre-adults are not the helpless grubs characterizing the social Hymenoptera. As a result, immature termites can function as workers. Furthermore, these immatures not only have the potential to follow several different ontogenetic routes-to workers, soldiers, reproductives and so on-but some basal taxa exhibit an amazing plasticity in that they can backtrack under the appropriate conditions, by molting to earlier, less differentiated forms, and then continue development along totally altered trajectories (Noirot 1990). Thus, worker termites have the potential to undergo a regressive molt and then begin to develop as a secondary reproductive. The second major distinguishing feature of the Isoptera is that they do not have the haplo-diploid sex determination system found in all Hymenoptera. Thus, all termite reproductives can lay both male and female eggs, and sisters and brothers are equally related. In the Hymenoptera, by contrast, many worker females cannot mate, and can therefore only lay male eggs. Also, if their queens only mated once, then the workers share, on average, 75% relatedness to their sisters. For these reasons, the evolutionary dynamics within termite and hymenopteran colonies are predicted to be quite different (Hamilton 1964).

Still, caste differences in ovariole number are features that many termites share with the majority of social hymenopterans. And, as in many hymenopterans, caste in termites is determined environmentally rather then genetically (Noirot 1990). The termite family Termopsidae is often proposed as the prototypical ancestral termite (Thorne and Traniello 2003), despite the fact that phylogenetic analyses robustly place the families Mastotermitidae and Hodotermitidae, respectively, as the most basal taxa within the Isoptera (e.g. Eggleton 2001; though one topology places the Hodotermitidae as the sister group to the Termopsidae). The justification for this unconventional character analysis is that the Mastotermitidae and Hodotermitidae are widely considered to be highly derived in their social organization (Thorne and Traniello 2003). For example, the Mastotermitidae and Hodotermitidae have more rigid castes than do the Termopsidae and other presumed basal families (reviewed in Thorne 1997). I will tentatively follow Thorne and

Traniello's suggestion, while expressing reservations about the cladistic relevance of their hypothesis.

Female termite workers develop along two different pathways: alate (the sexual forms, in which wings or wing buds are present) and non-alate (workers with no wing buds). Termopsids are characterized by extreme plasticity in reproductive tasks, even within non-alates. All castes, except possibly soldiers, retain the capacity to develop as either workers or reproductives (reviewed in Thorne 1997). And in *Archotermopsis*, soldiers have gonads that are as fully developed as mature alates (Imms 1920). If a termite colony has healthy primary reproductives, then they suppress reproduction in other castes through pheromones. A non-alate termite that becomes reproductive is known as a secondary reproductive.

A unique feature of secondary reproductives, at least in the termopsid *Zootermopsis angusticollis*, is that they increase their ovariole numbers as they begin to develop along the reproductive trajectory. [Note that these reported increases in ovariole number were judged at a stage when maturation had begun; thus the authors cannot distinguish between an actual increase in ovariole number and an activation of pre-existing "filamentous" ovarioles.] In one experiment (Brent and Traniello 2001b), secondaries increased their numbers of ovarioles by approximately 25% in 30 days (from 26 to about 32 ovarioles) when housed with non-alate workers and one reproductive female in experimental colonies. Primary (adult stage) reproductives placed in the same conditions underwent no change in ovariole number (approximately 31 ovarioles throughout), as would be expected for insect adults in general. In an even more dramatic experiment (Brent and Traniello 2001a), secondaries housed with one male and 6 non-alate workers increased their ovariole numbers by almost 50% in 60 days (to 38 ovarioles), while primaries again underwent virtually no change in ovariole number. Still, the primaries had greater egg laying rates under all conditions, suggesting a greater rate of öocyte maturation (see more on this below). In any case, these experiments demonstrate that termites can adjust their ovariole numbers in a seemingly adaptive fashion depending on colony condition.

Interestingly, termopsids have small colonies inhabiting decaying wood and do not leave their nest to forage (Thorne and Traniello 2003). These features may or may not be ancestral features for termites, but certainly provide a plausible explanation for their lack of caste rigidity. Once again, we see here a situation where a presumed relaxation of the ovarian growth *vs*. foraging function trade-off leads to an increase in reproductive potential.

And as West-Eberhard has pointed out (1978, page 853), "as long as a female has 'hope' of laying eggs...her participation in the worker tasks can be viewed as possibly or partially an investment in her own reproductive future." Clearly, the smaller the colony, the greater the chance that such a hope will be fulfilled (Thorne and Traniello 2003). This argument not only provides a compelling account for termopsid workers' acceptance of their non-reproductive status, despite their state of reproductive readiness, but also indicates a most plausible scenario for how sociality could have arisen in the first place from non-social ancestors. In fact, there is good reason to believe that such a scenario has played itself out repeatedly in the evolution of many of the independently derived, social taxa.

Honeybees

Honeybees (family Apidae, group Apinae) have some of the most spectacular instances of queen-worker differences in ovariole number (reviewed in Michener 1974, Ruttner 1988). These differences are entirely due to phenotypic plasticity: depending on the food allotment, a given female larva can develop as either a queen (with up to two hundred ovarioles per ovary) or a worker (generally with fewer than 10 ovarioles per ovary). In addition, workers are not endowed with the full reproductive system of queens, and thus can only lay unfertilized (male-determined) eggs. As I will discuss below, maturation in worker ovaries is repressed by the presence of a queen, but workers still have the capacity to reproduce. Still, even in the absence of a queen, a reproductive worker is quite limited in her reproductive output relative to queens, owing to her greatly reduced number of ovarioles.

The mechanisms underlying queen worker differences in ovariole number have recently begun to be elucidated. In the fourth instar larvae, in both queens and workers of the European honeybee *Apis mellifera carnica*, the ovaries contain over one hundred incipient ovarioles. But during the final stage and the lead up to metamorphosis, worker ovaries undergo massive cell death, which ultimately removes the great majority of these incipient ovarioles (Hartfelder and Steinbrück 1997; Reginato and Cruz-Landim 2002, 2003). Hormonal studies indicate that juvenile hormone levels in larval honeybees, which are higher in incipient queens than in workers, may underlie these morphogenetic differences (Rachinsky et al. 1990; Schmidt-Capella and Hartfelder 1998, 2002; reviewed in Hartfelder and Engels 1998).

Although this queen-worker ovariole number disparity holds for all honeybees, there is substantial variation in worker ovariole numbers in

different honeybee species and races (Table 3). Perhaps the most intriguing situation in honeybee reproduction comes from the cape honeybee, *Apis mellifera capensis*. It has been known for some time that this bee is unique among honeybees (and indeed all social Hymenoptera) in that the workers are capable of laying female eggs by thelytokous parthenogenesis (Onions 1912). Clearly such a situation creates a different social dynamic within the hive, since workers have the potential of winning the reproductive jackpot by giving birth to a future queen. Still, this ability has until recently only warranted a footnote in accounts of honeybee reproduction, as this unique bee is restricted to a small region in the very southern tip of South Africa. In fact, at one point, it was suggested that the dominant, aggressive southern African honeybee race, *Apis mellifera scutellata* (the bee race that begot the Africanized bee when transported to Brazil), was destined to overrun the poor, docile *capensis* bees (Ruttner 1977). Ironically, the reverse has happened. In recent years, a single clonal lineage of *capensis* bees has arisen that has the capacity to invade *scutellata* colonies, as described in more detail later in the chapter.

In queenright capensis hives, the queens are able to maintain some semblance of reproductive dominance. But even here, the workers are more uppity than in a typical *A. mellifera* colony, laying substantial numbers of eggs even in the presence of the queen pheromone (Moritz et al. 1999, Pirk et al. 2002). The possibility that the larger ovaries, themselves, are responsible for this difference in capensis is an intriguing one that remains untested. Interestingly, the trend in other honeybee species provides some support for this suggestion. As Table 3 shows, the queen-worker differences in ovariole number are far less dramatic in *A. cerana* than in typical *A. mellifera* races, and as we shall see, *A. cerana* workers have greater reproductive capacity than typical members of the genus. Furthermore, *A. dorsata* have the least profound worker-queen ovariole number differences among honeybees (Table 3), and have been described as having "the least pronounced caste dimorphism" in the genus (Engels and Imperatriz-Fonseca 1990, page 212). Recent work on Africanized honeybees in Brasil showed that worker bees with greater ovariole numbers and more active ovaries came from identifiable, genetic "patrilines" that dominated in new drone rearing after queen removal (Makert et al. 2006). A second study with *A. mellifera* showed a strong connection between progression through worker tasks (such as age at first foraging), pollen *versus* nectar foraging, and both ovariole number and degree of ovarian maturation state (Amdam et al. 2006). Furthermore, endocrinological variation among genotypes underlies these differences

Table 3 Differences in ovariole numbers between queens and workers in honeybee species, and in different races of *Apis mellifera*. Indicated are mean numbers of ovarioles per ovary (reported ranges in parentheses). Asterisks indicate range data for workers that was calculated differently from the other races/species: shown here are ranges of mean ovariole number values across the geographic ranges of *A. m. scutellata* and *A. m. capensis* in South Africa, as reported by Hepburn and Radloff (2002). Note that the numbers throughout the table were obtained in a variety of different conditions (temperature, seasonal, wild versus cultivated colonies, etc.), and not all of the *Apis mellifera* data is from colonies in their native area. Thus, the reader is cautioned from making too much of particular, small differences among races and species. Data from Alpatov 1938, Velthuis 1970, Michener 1974, Woyke et al. 1974, Weiss 1975, Buys 1988, Ruttner 1988, Koeniger et al. 1990, Dedej et al. 1998 and Hepburn and Radloff 2002. *A. andreniformis* data also from N. Koeniger pers. comm. Locality information from Ruttner (1988) and Wongsiri et al. (1996).

species	*native locality*	*queen ovariole number*	*worker ovariole number*
Apis mellifera adansonii	Central Africa	?	(1-11)
Apis mellifera carnica	Austrian Alps, northern Yugoslavia, Danube valley	175 (146-204)	(2-11)
Apis mellifera caucasica	Caucasus region	175 (169-181)	?
Apis mellifera lingustica	Italy	172.5 (155-190)	3.3 (1-24)
Apis mellifera mellifera	Central to southern Russia (Balkans to NE Mongolia)	162 (127-183)	5.3 (1-12)
Apis mellifera scutellata	Southern Africa	140 (136-149)	(2-5)*
Apis mellifera capensis	Cape region, South Africa	139 (127-151)	(9-18)*
Apis florea	Red Sea region, India, Bangladesh, Burma, Yunnan Province (China), southern Vietnam, Cambodia, Lao, Thailand	?	?
Apis andreniformis	NE India, Yunnan Province (China), Thailand, Lao, southern Vietnam, Malaysia, Sumatra, Java, Borneo, Palawan	48 (36-52)	2?
Apis cerana	Afghanistan, India, Pakistan, SE Asia, China, Korea, Japan, Indonesia (west of New Guinea), Philippines	73	8.6 (4-21)
Apis dorsata	India, Pakistan, SE Asia, Indonesia (west of New Guinea), Philippines	130	33 (17-60)

(Amdam et al. 2007). The implications of these data are profound: the size of the ovary, which is at center stage in the queen-worker social distinction in honeybees, is also intimately connected to the endocrinological status of the adult workers through genetic factors acting on ovary development during the larval phase [a connection also suggested by Sledge and colleagues' (1999) experiments on Ponerine ants described above, as well as Cepeda's (2006) studies on stingless bees (Apidae: Meliponini)]. We will revisit these studies below when considering the multiple evolutionary origins of eusociality among the Hymenoptera.

Which Stage-hands Set the Stage? The Genetic Determination of Ovariole Number

Not surprisingly, almost all work on the genetic determination of ovariole number comes from studies with *Drosophila melanogaster* and its close relatives. Because of the substantial (up to two-fold) intra-specific variability in ovariole numbers within *D. melanogaster* (Pappas and Engstrom 1974), and the even greater scope of interspecific variability within the melanogaster species group (see Table 1; Figure 2), this approach has proved and will continue to prove fruitful. Intraspecific hybridization experiments in *D. melanogaster* suggest that loci affecting ovariole number are concentrated on both of the large autosomes (chromosomes 2 & 3) rather than the X chromosome (Coyne et al. 1991; Chakir et al. 1995). Jones' (2004) interspecific hybridization studies showed that all chromosomes (especially chromosome 2 and the X chromosome) contribute to the lower fecundity of *D. sechellia*[9] when compared to *D. simulans*. However, unlike the Coyne et al. and Chakir et al. studies, Jones mapped differences in egg production, thus identifying factors involved in the lower öocyte maturation and/or oviposition rates in *D. sechellia*. So, it seems, there is not a tremendous amount of overlap between the genes involved in ovariole number and those involved in öocyte maturation/oviposition. Given the substantial dissociability among these different processes, both evolutionarily and developmentally, as I will describe throughout this chapter, this seems hardly surprising.

Initial QTL mapping studies indicated that a thankfully small number of loci might contribute the vast share of the heritability component in ovariole number differences among laboratory-selected lines of *D. melanogaster*

[9] *D. sechellia* are endemic to the Seychelles Islands, and are specialists on a fruit (*Morinda citrifolia*) that is toxic to other drosophilids. They also have the lowest ovariole number in the *melanogaster* species group (see Table 1; Figure 2).

(Wayne and Mackay 1998; recent studies might suggest otherwise, Bergland et al. submitted). The chromosomal regions with the predominant effects on ovariole number were further narrowed (Wayne et al. 2001), and 34 candidate loci in that region have now been identified (Wayne and McIntyre 2002). Two of the most noteworthy genes among these loci are *yellow-f* and *Actin87E*. The predicted *yellow-f* protein sequence is only similar to one known group of metazoan proteins: the honeybee (*Apis mellifera*) royal jelly proteins (approximately 30% amino acid identity; Maleszka and Kucharski 2000; Malecová et al. 2003). Because royal jelly seems to be important for the development of caste specific (including ovariole number) differences between queen and worker honeybees (Beetsma 1979), this finding for *Drosophila* raises the exciting possibility that the royal jelly proteins may have a common function in ovarian development in these divergent insect taxa. Still, that function would have been substantially modified in honeybees, where the proteins are obtained by the larvae through feeding by nurse bees, and are apparently involved in differential cell death in worker ovarioles (see Hartfelder and Steinbrück 1997), a process not seen in *Drosophila* ovarioles (Hodin and Riddiford 2000a). Actin, too, has been implicated in queen-worker ovariole number differences in honeybees (Schmidt-Capella and Hartfelder 2002), and *Actin87E* is one of several *Drosophila* actin genes. Further comparative studies are clearly warranted to determine whether these similarities are due to parallel evolution or common ancestry of ovariole determination mechanisms.

Simple experiments [using techniques such as those that we presented in Hodin and Riddiford (2000a)] with these 34 candidate loci from the laboratory lines would substantially increase the likelihood of identifying given genes that are involved in ovariole number variability in natural populations, or even differences due to plasticity. In other words, if mutations in these candidate loci phenocopy specific ontogenetic differences (either plastic or genetically-fixed) that we identified in 2000 (see Table 1), then those loci would be of particular interest. For example, partial loss of function mutations in the ecdysone receptor (*EcR*) and ultraspiracle (*usp*) genes, whose protein products dimerize to form the insect ecdysteroid receptor, phenocopy one of the mechanisms (mechanism "**d**" in Table 1) accounting for the lower ovariole numbers (relative to the Sevelen strain of *D. melanogaster*) in *D. sechellia*, *D. mauritiana, D. yakuba,* the Honduras strain of *D. simulans* and the Nahal Canyon strain of *D. melanogaster* (Hodin and Riddiford 1998, 2000a). Indeed, one of the epistatic QTLs identified by Bergland et al. (submitted) includes the EcR gene (A. Bergland, pers. comm.).

Intriguingly, Malecová et al. (2003) identified USP binding sites in the 5' untranslated regions of all five of the honeybee royal jelly protein genes, directly upstream of their translational start sites. Such USP binding sites have been proposed as possible JH-mediated USP regulatory sequences, distinct from the classic EcR/USP-mediated ecdysteroid binding sites (Jones and Sharp 1997). Indeed, methyl farnesoate (MF), a biosynthetic precursor of JH, binds USP 150x more strongly than does JHIII (Jones et al. 2006). This finding raises the exciting possibility that MF could be a bona fide ligand for USP. USP, in the absence of EcR, is expressed at high levels in the differentiating terminal filament cells of the larval ovary in *D. melanogaster* (and its sister species as well; Hodin and Riddiford 2000a), and alterations in this terminal filament expression of USP results in defects in ovariole morphogenesis and reduction in ovariole number (Hodin and Riddiford 1998). These results could provide the first evidence of a direct link between the nutritional (yellow/royal jelly proteins) and hormonal (ecdysteroid/JH/MF) regulation of ovariole number. Recent studies suggest that insulin-like signaling may also be directly involved in this apparent link between nutrition and hormonal regulation (Tu and Tatar 2003; Flatt et al. 2005), and a recent QTL study of ovariole number differences between *D. simulans* and *D. sechellia* found that the genomic region of largest effect contains the *insulin receptor* (*InR*) ortholog in *D. simulans* (Orgogozo et al. 2006). Bergland et al. (submitted) have also identified QTL's with large effect on ovariole number that contain genes involved in insulin and related signaling pathways. Indeed, mutations in insulin pathway genes in *D. melanogaster* result in reduced ovariole numbers (Tu and Tatar 2003; Richard et al. 2003).

Thus, with these recent developments, we are tantalizingly close to being able to determine if the genetic factors that are involved in inter- and intra-specific ovariole number differences are also involved in ovariole number plasticity. Not only would such information add tremendously to our understanding of reproductive plasticity in insects, but it would be groundbreaking for our general understanding of the evolution of phenotypic plasticity as well.

So Ovariole Numbers Are Set: Now What? Plasticity in Öocyte Maturation

In all insects except coccids (see above), maximal ovariole number is fixed by adult eclosion, and sometimes much earlier. However, plasticity in reproductive potential hardly ends here. In fact, modulation in the rate of

öocyte maturation is probably the predominant mechanism by which insects attempt to optimize their reproductive output to suit environmental conditions (Labeyrie 1978). Many different external stimuli are known to modulate the rates of öocyte maturation in different insects: temperature, food availability, day length, the presence of mates, mating, the availability of ovipositional resources, oviposition itself, the presence of dominant reproductives (in social insects), and so on (reviewed in Labeyrie 1978, Wheeler 1996, Hopkins and Ekbom 1999, Tammaru and Javois 2000, Papaj 2000, Jervis et al. 2005). This is eminently sensible. Many organisms, including insects, are known to trade off current versus future reproduction (reviewed in Hopper 1999) and to vary substantially in degree of parental investment per offspring (e.g. Halffter and Edmonds 1982, Tallamy 1984). This particular trade-off can be more serious than just a timing of reproduction issue: reproduction in a diversity of insects induces a direct cost to life expectancy (Partridge et al. 1987, Tatar and Carey 1995, Sgro and Partridge 1999; Herman and Tatar 2001, Jervis et al. 2005, Flatt and Kawecki 2007) *via* a nutrient allocation mechanism (Tatar and Carey 1995). Also, such allocation trade-offs vary within taxonomic groups among species with differing life histories (e.g. Stevens et al. 2000, Emlen 2001) suggesting that such trade-offs are moldable by natural selection. Furthermore, not only is the production of eggs energetically costly, but the extra weight imposed by a fully mature ovary can have substantial mechanistic consequences as well, such as speed of anti-predator escape or lift in flying insects (Berrigan 1991). Therefore, insects under variable conditions would be expected to adjust their processes of öocyte maturation according to the suitability of the environment. The goal, of course, would be for a female to produce the maximum number of eggs in her lifetime that have the maximum chance of surviving to adulthood (within the parameters of ontogenetic and other constraints, of course). There are many excellent examples that suggest that this is precisely what insects attempt to do.

Plasticity in the timing of öocyte maturation has been adequately reviewed on numerous occasions (see above). Still, most (though not all) of these reviews have focused on mainly one of two areas: ecological stimuli influencing öocyte maturation, or the mechanisms (hormonal and otherwise) controlling the maturation process. My purpose here is not to attempt a comprehensive review of this unwieldy subject. Instead, I intend to illustrate, with specific examples, four points: 1) different insects use different environmental cues to modulate öocyte maturation in a seemingly adaptive fashion; 2) öocyte maturation entails trade offs with somatic

functions (the most famous is flight, the so-called "ovary flight syndrome"); 3) the internal mechanisms regulating öocyte maturation are numerous, and vary even within closely related groups; and 4) a thorough understanding of plasticity in insect reproduction will only come from a holistic approach, combining ecology and ontogenetic mechanisms in a phylogenetic context. I will begin this section by examining specific examples of plasticity in öocyte maturation in a wide diversity of insect taxa. I will conclude the section with some generalized thoughts concerning patterns (or lack thereof) in the control of öocyte maturation in insects.

Perhaps the most typical, and intuitively obvious, influence on öocyte maturation is nutrition (Labeyrie 1978). Many insects eclose with substantial stored materials left over from pre-adult development. In such cases, it is not uncommon to find adults that eclose with öocyte maturation underway, or even complete. We can make several predictions concerning selective regimes that might favor such a reproductive strategy: 1) plentiful food resources in the larval environment; 2) lack of larval competition; 3) low larval predation and/or parasitization rates; 4) lack of adult feeding and/or short lived adults; 5) typically poor or ephemeral adult food resources; 6) typically heavy predation pressures on adults; 7) intense competition for oviposition sites; 8) high probabilities of finding a mate quickly; and 9) oviposition sites nearby (or identical with) the larval habitat that the individual just left. Several of these hypotheses have not, to my knowledge, been rigorously tested, though examples of others abound (reviewed in Labeyrie 1978, Papaj 2000).

Blowflies Need Protein

For those insects that neither eclose with substantial reserve materials, nor have mature ovaries at eclosion (as well as those insects, such as mosquitoes, that will mature multiple batches of eggs in succession), adult feeding is often the stimulus for öocyte maturation (reviewed in Wheeler 1996, Papaj 2000). In such cases, proteins are often limiting, since excess proteins are needed for the synthesis of vitellogenins (the major class of yolk proteins produced by the fat body and transferred to the öocytes during vitellogenesis). Thus, protein intake (feeding) is often the direct trigger for öocyte maturation.

Adult sheep blowflies, *Lucilia sericata* (Diptera: Calliphoridae), are short-lived (less than a week, though they can survive longer in captivity) and cannot initiate vitellogenesis until they find a source of protein, such as a

live sheep, carrion, or even manure. Barton Browne et al. (1979) and Wall et al. (2002) fed females liquid liver in different concentrations, and noticed that öocyte maturation seemed to pass through at least two protein threshold stages. At the lowest protein levels, vitellogenesis began in all ovarioles (stage 1) but was not completed.[10] At moderate levels, vitellogenesis continued (stage 2), but many follicles arrested (their development could be reinitiated) or were resorbed, allowing the materials to be reused by the female, perhaps for somatic maintenance. At high levels, the majority of the follicles matured, although some were still resorbed. The authors suggest that these responses, where the flies continually assess their nutritional state at multiple stages, and modify öocyte development accordingly, are bet-hedging strategies regarding possibilities of finding further protein resources. In protein limited individuals, it would be disadvantageous to attempt to mature too many eggs, since if no further protein sources are found, effective reproduction may be impossible in these short lived adults. Moderately fed individuals can remain at a later resting stage, to take full advantage of possible additional resources, if they are found. Another (not mutually exclusive) explanation is that the bet-hedging strategy will allow them to mature the maximum number of eggs at one time under any given feeding regime, since the adults will hold and then lay essentially all of their eggs at once (independent of the egg load) once an appropriate host is located (Barton Browne et al. 1990). And for *Lucilia*, that would likely be their one and only opportunity for oviposition.

In accord with this ecological account for the multi-stage maturation process in *Lucilia* is the observation that this species apparently starts to mature all of its primary öocytes simultaneously in every ovariole (Barton Browne et al. 1979). While this ability clearly allows for the rapid, synchronous maturation of a complete batch of eggs, it may also impose a constraint upon öocyte maturation, such that a miscalculation of maturational timing could be disastrous for their chances of rearing successful offspring. Thus, the multi-stage maturational process of *Lucilia* might be best understood as a bet-hedging life history strategy in the context of constraints relating to their particular mechanics of synchronic öocyte maturation.

[10] Such maturational synchrony across all ovarioles is a common feature among several disparate so-called "anautogenous" species of dipterans (Barton Browne 2001). Such a situation, which is not by any means universal among insects as a whole, may be considered a developmental constraint in *L. sericata* that could help explain their threshold pattern of öocyte maturation.

Lubber Grasshoppers and the Limits of Food-induced Plasticity

Lubber grasshoppers, *Romalea microptera* (=*guttata*) (Orthoptera: Acrididae), eclose as adults with immature ovaries, containing only early-stage öocytes in each of its ~ 65 ovarioles. Well-fed adults oviposit for the first time about one month later, while those with a reduced food ration (87% reduction in amount offered) delay first oviposition by an additional 2 weeks (Moehrlin and Juliano, 1998). Surprisingly, a switch from high to low food 14 or 21 days after eclosion did not result in a significant lengthening of time to oviposition. Thus, the reproductive cycle for lubbers can be described in terms of a plastic (early) and canalized (late) phase (Juliano et al. 2004). The switch from the plastic to the canalized phase corresponds to a transient rise in the titers of juvenile hormone (JH) (Hatle et al. 2000). Although no direct connection has been demonstrated between this rise in JH and the reproductive events that follow, these results suggest that the release of JH may initiate a chain of events involved in the control of reproductive timing, which can no longer be modulated by external conditions. By contrast, number of öocytes being developed remains plastic throughout the reproductive cycle (see below), so the reproductive process as a whole is not canalized, just the timing of reproduction.

In sum, plasticity in timing seems to be constrained late in the oviposition cycle in lubbers, though at different times in different populations (Hatle et al. 2002). A parallel process of canalization in the timing of the last molt in lubbers (Hatle et al. 2003b) might indicate that there is something in general about the hormonal control of development in this species that constrains timing of developmental events (the similarities in the ovipositional and ecdysis behavioral networks in insects may be related to this apparent constraint; see Figure 7, below)

This canalized (or constrained) ontogenetic trajectory contrasts sharply with the highly plastic reproductive trajectory in the sheep blowfly (Diptera: Calliphoridae) discussed above. What is the nature of this difference? Does the apparent use of JH as an öogenic regulator in lubbers impose this constraint in timing? If that is the case, then one would need to account for the extreme variation in the functions of hormones in insect reproduction in different insects, including orthopterans (Strambi et al. 1997). Interestingly, vitellogenesis in black blowflies (*Phormia regina*, also in the family Calliphoridae), as in most dipterans, seems to be under the control of ecdysteroids rather than JH (Yin and Stoffolano 1997), while ecdysteroids are probably not involved in vitellogenesis in lubbers (Hatle et al. 2003a). Indirect evidence suggests that ecdysteroids might also be responsible for

the threshold stage progression described above for the sheep blowflies (A.D. Clift 1972 Ph.D. thesis, cited in Barton Browne at al. 1979), as has been shown at least in part for other so-called "anautogenous" dipterans: those in which their öocytes mature synchronically, such as *P. regina*, mosquitoes (family Culicidae) and the house fly (family Muscidae) *Musca domestica* (Barton Browne 2001). Still, it would be far too cavalier at this stage to suggest any connection between these differences in JH/ecdysteroid control and relative plasticity in ovary maturational processes in blowflies and lubbers.

Several key pieces of information, including detailed studies on the ovarian events occurring during the plastic and canalized phases in lubbers, as well as more detailed hormonal studies on both lubbers and sheep blowflies, would go a long way towards an understanding of the mechanistic underpinnings of these differences in reproductive patterns. Furthermore, selection experiments could allow one to distinguish between ontogenetic constraints and stabilizing selection on these reproductive processes. Finally, detailed intra- and interspecific comparative studies on related groups of grasshoppers (extending on Hatle et al 2002) and blowflies, as well as other anautogenous and autogenous dipterans, would offer a much needed comparative focus.

Cross-continental Insect Migrations and the Ovary-flight Syndrome

In examining various instances of reproductive plasticity in insects, one pattern has appeared repeatedly: an apparent trade-off between flight and reproduction known as the "ovary-flight syndrome." Whether this trade off is energetic (i.e. conflicting metabolic demands) or biomechanic (i.e. lift production) in nature (or, more likely, both), the trade-off clearly imposes substantial constraints on insect life histories. A cogent example for the negative relationship between dispersal and reproduction is the process of absconding in honey bees, typically a plastic response to deteriorating conditions (due to weather, resource availability, parasites, etc.). When absconding, the entire colony abandons its nest site, leaving behind mainly empty combs, and re-establishes at a new, suitable location. In preparation for this transition, the mature queen has to regress her ovaries in order to fly. While absconding is rare among the European races and the European-derived honey bees introduced to North America, it is commonplace among honey bees of the tropics and subtropics (Spivak et al. 1991).[11] Thus, for

[11] In fact, this propensity to abscond may be considered a preadaptation (see footnote 8, above) for the invasive behavior of the infamous Africanized bees of Brasil.

example, a given African honey bee queen from a particularly mobile colony might mature and regress her ovaries several times throughout her lifetime.

The classic examples of the ovary-flight syndrome, however, involve migratory insects that disperse at most once in their lifetimes, and generally at a defined stage. Several excellent reviews have described the ovary-flight syndrome in particularly well-studied migratory taxa, such as solitary and gregarious locusts (*Locusta migratoria*; Applebaum et al. 1997), crickets (*Gryllus* spp.; Zera and Harshman 2001), the army worm *Pseudaletia unipunctata* (McNeil et al. 1996), the soap-berry bug *Jadera haematoloma* (Dingle and Winchell 1997) and the water strider *Limnoporus canaliculatus* (Zera 1985). Here I will focus on what is perhaps the most famous of all migrating insects, the monarch butterfly *Danaus plexippus*. Clearly any insect that accomplishes a yearly migration from Canada to Mexico must have nearly optimized both energetics and lift production, and thus would make an excellent case study for investigating the ovary-flight syndrome. Furthermore, while many papers have been written on monarch migration, ontogeny, physiology and reproduction, I am aware of no attempts to synthesize these varied aspects of monarch biology in the explicit context of reproductive-flight trade-offs.

Although the spectacular southern migration of monarchs in autumn alluded to above is accomplished by individual insects that fly the entire route, the northward migration the following spring is more of a stepping-stone process (reviewed in Brower and Malcolm 1991). The eastern North American monarchs (the population that stretches from the Rocky mountains to the Atlantic ocean) migrate south and overwinter in the highlands north of Mexico City. In spring, these same individuals migrate north to their summer breeding grounds in the southern United States, and reproduce and die there. Several weeks later, their offspring continue the migration north. In the Mexican overwintering sites, the ovaries of female monarchs remain in an immature state (Barker and Herman 1976). So during their northward migration, the ovary is small, and energy is instead invested in the flight muscles and energetic stores. By the springtime, photoperiod and temperature interact synergistically to promote ovarian development; overwintering monarchs transferred to long day, warmer incubators will begin to mature öocytes (Barker and Herman 1976; Herman et al. 1989). Still, excessive temperatures inhibit reproduction, and by summertime, larvae are not found in the southern part of their range. Furthermore, the photoperiod and temperature levels which maximally promote öocyte maturation in monarchs are also the same levels that cue growth of their primary host plant, the milkweed *Asclepias syriaca* (Barker and Herman 1976). All of these factors presumably work together, resulting

in a continued northward migration into summer: bouts of reproduction are followed by sub-optimal reproductive conditions, leading to ovarian regression and further migration north.

Throughout the long days of summer, several non-migratory generations of monarchs are produced in the northern US and southern Canada. In fact, the lack of migration during this phase is not the only manifestation of the female monarch's ovary-flight trade-off. Activity patterns of male and female breeding monarchs show a striking difference: while males engage in longer flights, patrolling mating grounds and searching for females, female flights are limited mainly to shorter, less-frequent and slower plant-to-plant foraging trips (Zalucki and Kitching 1982). Thus, during the period of most intense monarch reproduction, öocyte maturation correlates with low flight activity.

Monarch adults eclose with only immature öocytes. Under winter conditions, the ovaries remain in this immature state, but summertime photoperiod conditions induce öocyte maturation within five days after emergence (Herman et al. 1981). This surge in öocyte growth under summer conditions is preceded by a rise in the JH titers in the hemolymph of newly eclosed adults (Lessman et al. 1989), which is required for öocyte development to proceed (Barker and Herman 1973, Herman 1975, Lessman et al. 1982).

Levels of JH also vary seasonally (Lessman and Herman 1983), with high female JH titers in June and July (reproductive generations), moderate titers in August to October (migrating generation) and low titers in November to March (overwintering generation).[12] Indeed, the low JH titers in overwintering monarchs has been recently proposed to be related to longevity, as JH injections into overwintering monarchs lower their life expectancy in much the same way as transfer of such butterflies to artificial summer-like conditions (Herman and Tatar 2001). High JH titers promote öocyte maturation in a wide variety of insects (see below), while moderate JH titers have been shown to induce migration in the milkweed bug *Oncopeltus fasciatus* as well (Rankin and Riddiford 1978). Removal of the corpora allata results in a drastic reduction in long distance flights in tethered monarchs, further supporting the involvement of moderate JH levels in monarch migration. Finally, increasing JH levels (by injection) in migratory adults can initiate öocyte maturation (Herman 1975).

[12] Note that these data are combined from two distinct populations of monarchs: the eastern population (wild caught in Wisconsin and Minnesota) in May to September, and the western population (from overwintering sites near San Francisco, California) in October to March. Note also that these latter titers were relative values derived from a *Galleria* wax test.

Intriguingly, a direct mechanistic connection has been suggested between flight activity and reduced öocyte maturation in monarchs. Monarchs injected with radioactively-labeled JH showed a rapid increase in JH metabolites following a 40-minute tethered flight relative to unflown controls (Lessman and Herman 1981). Increased thoracic temperature had the same effect, leading to the fascinating proposal that the increased thoracic temperature resulting from flight activity directly causes a reduction in JH activity (Lessman and Herman 1981). These authors suggested that the thorax acts as a "JH gauntlet" which the hormone passes through on the way to the ovary-containing abdomen. Under high activity conditions, JH would be broken down on the way to the abdomen, and öocyte maturation would fail to be activated.[13] Hence, in monarchs, physical activity may feed back to control reproduction via hormones. This idea that environmental temperatures directly control the concentration, timing, and effects of hormones, has great importance for phenotypic plasticity.

Finally, Rankin (1986) showed an inverse relationship between the number of mature eggs being held by a female monarch and the length and likelihood of continuous, tethered flight by the same female. Furthermore, monarchs with their corpora allata removed flew very little, but flew about two fold more when injected with JH. In sum, these results support the existence of a direct ovary-flight trade-off in monarchs, and further suggest that JH lies at the center of this trade-off.

Few attempts have been made to apply modern techniques in insect biochemistry, physiology and development to further test these and other hypotheses regarding the ovary-flight syndrome in monarchs. Recent work with the cricket *Gryllus firmus* has demonstrated a direct link between fatty acid metabolism and reproductive versus flight energetics (Zera and Zhao 2003a, Zera, this volume), and long wing and short wing *G. firmus* morphs have different energetic profiles reflecting their different propensities for migration *versus* reproduction (Zera and Zhao 2003b). An application of these approaches to long-distance migratory species such as *D. plexippus* would not only allow us to ascertain the generalities of the underlying mechanisms (such as those uncovered by Zera and Zhao) and their associated trade-offs, but would also aid in our comprehension of how the spectacular cross-continental migration of monarchs is physiologically possible.

[13] Incidentally, this negative relationship between flight activity and circulating JH titers has apparently been circumvented in the long-distance migrating grasshopper *Melanoplus sanguinipes*, where tethered flights to exhaustion are followed by a rapid rise in JH titers and rapid onset of öocyte maturation (Min et al. 2004). Such rises in JH were not found in unflown or briefly-flown controls.

Parasitoid Wasps: How Important Is Numbers of Eggs Being Held?

Life histories in parasitoid wasps (Hymenoptera: Apocrita) have been intensively studied, largely due to their usefulness in insect "pest" control. Substantial research has focused on understanding the conditions leading to egg maturation and successful reproduction in this group. Not surprisingly, a vast array of reproductive strategies is employed in the various parasitoid taxa. Jervis and colleagues (2001, 2003) conducted a broad comparative study of parasitoids, and made the surprising observation that body size is inversely correlated with "ovigeny index" (the proportion of eggs that are mature at adult eclosion), intraspecifically as well as interspecifically (thus it is likely a plastic response as well). Why would smaller wasps tend to have a greater proportion of mature ovaries at eclosion? Jervis and colleagues suggest the possibility that small size correlates with higher mortality rates (and thus shorter life expectancies) in the field due to increased risks of predation and/or desiccation. The relevant data on these points, however, are limited and equivocal.

Wang and Messing (2003) examined the stimulant for öocyte maturation in the braconid wasp *Fopius arisanus* (Hymenoptera: Braconidae), a fruit fly (Diptera: Tephritidae) generalist with a very low ovigeny index. During the days following eclosion, öocyte maturation proceeds independently of host stimuli, food and access to males in any combination. However, after this first batch of öocytes matured, the rate of maturation in subsequent öocytes was substantially increased only in females given access to host stimuli, food and males. An even greater increase in öocyte maturation was seen in wasps provided with the host and the host fruit (and were, thus, allowed to oviposit for the first time), even when starved. Thus, the first round of öocyte maturation seems to be an inherent process, largely independent of external cues. Further maturation, though, was most effective in response to oviposition, a maturational cue found in many parasitoid wasps and other insects (reviewed in Papaj 2000).

These results have three important implications. First, the apparent inverse proportionality between longevity and reproductive effort suggests that forgoing additional öocyte development allows for longer survival (reviewed in Papaj 2000). Second, the finding that oviposition was the predominant maturational trigger begs the following question: how does oviposition transmit the signal to initiate öocyte maturation? We will return to this point near the end of the chapter. Third, these results appear to contradict a hopeful assumption on the part of many parasitoid researchers:

that a fully mature ovary's "egg load" (the numbers of eggs held) is a general stimulus to oviposition in parasitoids (e.g. Mangel 1989). If this were true, it would greatly simplify pest management strategies, as releasing wasps with full egg loads would then enhance their success in bio-control. Unfortunately, the explanations for reproductive decisions of parasitoids will only be found, it seems, in the context of a detailed understanding of the underlying öocyte maturation mechanisms, which are likely to be quite variable across taxa (see also Jervis and Ferns 2004).

In another example, Rivero-Lynch and Godfray (1997) have shown that *Leptomastix dactylopii* parasitoids (Hymenoptera: Encyrtidae), when provided with plentiful numbers of their mealybug hosts, and thus unlimited opportunities to oviposit, have egg loads equivalent to host-limited and host-deprived wasps. It seems that the presence of plentiful hosts and/or oviposition leads to an increase in maturation rates (see Alonso-Pimentel et al. 1998 for an ingenious method used to distinguish among similar possibilities in walnut flies). In any case, egg load seems largely irrelevant to the reproductive biology of these encyrtid wasps as well.

Tephritid Fruit Flies: Comparative Approaches

For many of the same reasons indicated above for parasitoids, tephritid fruit flies (Diptera: Tephritidae), which are major fruit pests around the world, have been the focus of much research into reproductive life histories. Happily, many of these studies have involved a comparative approach, something which is decidedly lacking from the majority of studies in insect life histories.

One set of studies (Aluja et al. 2001; Díaz-Fleischer and Aluja 2003) involved a comparison of the reproductive biology of two species from the genus *Anastrepha*. *A. obliqua* oviposits on a wide variety of fruits, mostly from the family Anacardiaceae, which includes the cashew, mango and pistachio. *A. ludens* is more of a specialist on plants in the rue family (Rutaceae), including the yellow chapote (*Sargentia greggii*) and the white sapote (*Casimiroa edulis*). *A. obliqua* tends to lay a single egg on its high quality host fruits, which are, for the most part, abundant, synchronous and highly ephemeral. The lower quality host fruits of *A. ludens* are comparatively less numerous, less synchronous and less ephemeral, and *A. ludens* tends to lay eggs in batches of up to 40 eggs (Aluja et al. 2001). *A. ludens* are larger in size than *A. obliqua*, and have fewer ovarioles (22–25 vs. 30–33) (F. Díaz-Fleischer, personal communication).

Under similar dietary conditions, *A. obliqua* consistently matured more than twice as many öocytes than did *A. ludens*. In addition, the presence of host fruit volatiles led to substantial increases in öocyte maturation only in *A. obliqua* (Aluja et al. 2001). A second study (Díaz-Fleischer and Aluja 2003) examining lifetime oviposition in the two species can help account for these results. When offered a low, high or variable availability of hosts, *A. ludens* maintained a strikingly constant egg oviposition pattern. *A. obliqua*, by contrast, modulated its oviposition patterns to match host availability. It seems that the high egg loads are maintained in *obliqua* (and aided by their higher ovariole numbers) in order to take advantage of rare but high abundance host patches. *A. ludens* females are relatively non-selective about their oviposition choices, since their hosts tend not to appear in blooms in nature. While the mechanisms underlying the two different maturation trajectories remain to be elucidated, work on other insects suggests some plausible hypotheses, which we will return to at the end of this section.

A second comparative approach involving tephritids has focused on flies in the genus *Bactrocera* (formerly *Dacus*). The genus includes species with a wide range of reproductive ecologies, from specialists (e.g. the olive fly *B. oleae* and the solanum fruit fly *B. cacuminatus*) to generalists (e.g. the Queensland fruit fly *B. tryoni*) to intermediates (the cucumber fly *B. cucumis* and the Jarvis' fruit fly *B. jarvisi*). Such diversity within a restricted taxonomic group allows for a fairly rigorous test of a commonly-held hypothesis (e.g. Labeyrie 1978): namely, that generalist taxa (in which oviposition possibilities are abundant) tend to have less specific control mechanisms for initiating öocyte maturation than specialist taxa (where hosts are limited). The example cited above of the two *Anastrepha* species tends to support this hypothesis. In *B. oleae*, the ovaries are activated in the presence of olives, which, on the Hellenic island of Corfu, are available in May and late July, but not June through early July (Fletcher et al. 1978). *B. oleae* ovaries regressed in the field in July, and lab experiments showed that presence of fruits, as well as temperature and humidity conditions mimicking those in late July, lead to öocyte maturation (Fletcher et al. 1978).[14] So, it seems, this specialist fly has fairly specific (environmental)

[14] A correlated adaptive explanation here is that immature ovaries in the absence of appropriate hosts aid in dispersal by decreasing wing loading. Such a situation has been nicely demonstrated for the potato tuberworm *Phthorimaea operculella* (Lepidoptera: Gelechiidae), where development on tomato (a sub-optimal host) leads to lower rates of egg maturation and a greater tendency to fly, while development on potato (an optimal host) leads to higher maturation rates and a reduced tendency to fly (Coll and Yuval 2004).

control mechanisms that match its maturational timing with the presence of its preferred host fruit. But does this pattern hold in related species with different reproductive patterns?

Fitt (1986) deprived groups of *B. cacuminatus*, *cucumis*, *jarvisi* and *tryoni* of hosts for up to 16 days, and then offered either previously unacceptable or highly unpreferred fruit hosts (determined in choice experiments; different fruits for different species). He recorded numbers of eggs laid in oviposition tests conducted on various days after host deprivation when compared to undeprived controls. All flies were well fed on a sugar/yeast mixture. The specialist *B. cacuminatus* and intermediate *B. cucumis* laid virtually no eggs on unpreferred hosts even after 16 days of host deprivation. *B. jarvisi* did lay eggs on unpreferred hosts, but there were no differences between deprived and undeprived flies, and time since deprivation had no effect on numbers of eggs laid on these unpreferred hosts. By contrast, the generalist *B. tyroni* readily laid on an unpreferred host (the wild tobacco *Solanum mauritianum*), but only when host-deprived for 4 days or more.

These differences in oviposition under host-deprived condition were mirrored by egg load measurements (Fitt 1986). *B. cacuminatus* (a specialist) showed no difference in egg load between deprived and undeprived flies, deprived *B. cucumis* and *B. jarvisi* (intermediates) had double the egg load of undeprived flies, and deprived *B. tryoni* (a generalist) had 3–5 times the egg load as undeprived flies. Interestingly, the egg loads in the three non-generalist species never exceeded one egg per ovariole, while egg loads in *B. tyroni* were up to twice the ovariole number. Given the positive correlation between ovariole number and number of host plant species across 14 *Bactrocera* species (Fitt 1990), it seems that generalist taxa use several tricks to increase their potential reproductive output. However, the full significance of these results will only be clear when more specialist and generalist *Bactrocera* (and *Anastrepha*) species (there are hundreds) are examined in a phylogenetic context.

Hormones and Insect Reproductive Variability and Plasticity: Nothing New Under the Sun

It is fair to say that the major lesson learned from a half-century of comparative studies on insect development is that hormones are involved in just about every ontogenetic process you can think of (Nijhout 1994). Still, this ubiquity of hormonal involvement in insect development is paradoxical: how is the extreme variability in insect life cycles, morphology, physiology and behavior controlled by what is largely a highly stereotyped

pattern of ontogenetic hormonal profiles? One answer comes from the apparent modularity in hormonal response indicated by tissue- (and cell-type-) specific patterns of hormone receptor expression. Evidence for such evolutionary variation has been presented (e.g. Hodin and Riddiford 2000b) or is suggested (e.g. Rountree and Nijhout 1985a,b) by studies in a wide variety of insects. A second answer involves the observation that hormonal profiles are much more variable in adults than in pre-adult stages (Nijhout 1994). A third answer may relate to the fact that hormonal release in some cases is directly triggered by nutritional inputs (reviewed in Adams 1999; Barton Browne 2001), demonstrating the physiological basis for the connection between nutritional intake and reproductive plasticity. Still, the extreme variability in hormonal control of reproduction across insects defies an easy characterization. I'll begin by presenting some evidence for this extreme variability, and end with some speculation concerning evolutionary scenarios that could have generated such a variable system.

Table 4 gives an indication for some of this variability. While JH promotion of vitellogenesis and öocyte maturation is fairly widespread (and has, thus, been often proposed to be an ancient function in insects; e.g. Bellés 1998), this pattern is by no means universal.[15] For example, JH inhibits vitellogenesis and/or vitellogenin (VG) synthesis in the sweet potato weevil *Cylas formicarius*, the gypsy moth *Lymantria dispar* (where VG synthesis apparently depends on falling JH titers) and, possibly, the western tent caterpillar *Malacosoma pluviale*. There are also many taxa in which JH seems to have no role at all (or, at least, a drastically reduced function; Barchuk et al. 2002) in vitellogenesis (such as most ants and the eusocial honeybees and stingless bees, as well as the silk moth *Hyalophora cecropia*), and those in which JH effects seem to be restricted to late- or post-vitellogenic stages (such as the tobacco hawk moth *Manduca sexta* and the apple codling moth *Cydia pomonella*). As is obvious from Table 4, the reproductive functions of ecdysteroids are, if anything, more variable than those for JH. This table is not a comprehensive list of all insects for which hormonal effects on reproduction have been studied, but gives a flavor for the diversity in hormonal control mechanisms in insects. As such, it overemphasizes the

[15] I use the term "JH" here and throughout to refer collectively to all of the different forms of juvenile hormone (JHI, JHII, JHIII, JH-bis-epoxide, etc.) that have been shown to be active in various insects groups. I also have not distinguished here among experiments in which, for example, corpora allata have been removed versus perhaps less convincing hormone manipulation methods. My apologies to my former advisor and all other JH enthusiasts for this oversimplification. Interested readers can find more detailed information in the papers to which I have referred herein.

Table 4 Vitellogenesis control mechanisms in selected insects. Information comes from a wide variety of sources and experimental evidence. Question marks indicate mostly correlative data (such as a correlation of whole body hormone titers with the timing of vitellogenesis), while mechanisms without question marks derive from manipulative experiments (either pharmacological or manual removal of hormone sources and/or ectopic hormonal applications). Blanks mean that the functions of this hormone in insect reproduction have not, to my knowledge, been investigated. References (some of which are reviews, others are experimental studies) as follows: [a]Taub-Montemayor et al. 1997; [b]Ram et al 1988; [c]Scott et al. 2001; [d]Rankin et al. 1997; [e]Stay et al. 1980; [f]Schal et al. 1997; [g]Yin and Stoffolano 1997; [h]Klowden 1997; [i]Bownes 1989; [j]Audit-Lamour and Busson 1981; [k]Hodin and Riddiford 1998; [l]Davey 1997; [m]Hartfelder et al. 2002; [n]Bloch et al. 2002; [o]Sommer and Hölldobler 1995; [p]Robinson and Vargo 1997; [q]Ramaswamy et al. 1997; [r]Webb et al. 1999; [s]Fescemeyer et al. 1992; [t]Zeng et al. 1997; [u]Shaaya et al. 1993; [v]Nijhout and Riddiford 1974, 1979; [w]Applebaum et al. 1997; [x]Strambi et al. 1997; [y]Bradley et al. 1995; [z]Bellés 1998.

Order	*Family*	*Species*	*Function of JH in female reproduction*	*Function of ecdysteroids in female reproduction*	*Ref.*
Coleoptera	Chrysomelidae	*Leptinotarsa decemlineata*	promotes but not necessary for VG synthesis or oviposition	no effect on VG synthesis	a
Coleoptera	Coccinellidae	*Coccinella septempunctata*	promotes VG synthesis		a
Coleoptera	Curculionidae	*Anthonomus grandis*	promotes VG synthesis in pre-reproductive but not in diapausing females; not involved in yolk uptake or oviposition	no effect on VG synthesis in diapausing females; not involved in oviposition	a
Coleoptera	Curculionidae	*Cylas formicarius*	inhibits vitellogenesis?		b
Coleoptera	Silphidae	*Nicrophorus orbicollis*	promotes öocyte maturation?; inhibits oviposition; promotes maternal care?		c
Dermaptera	Carcinophoridae	*Euborellia annulipes*	promotes reproductive behavior; promotes maturation; inhibits brood protection	inhibit brood protection	d

(Contd.)

Table 4 (Contd.)

Order	*Family*	*Species*	*Function of JH in female reproduction*	*Function of ecdysteroids in female reproduction*	*Ref.*
Dictyoptera	Blaberidae	*Diploptera punctata*	promotes VG synthesis and öocyte maturation; inhibits ovulation	Inhibit JH production preceding ovulation	e
Dictyoptera	Blattellidae	*Blatella germanica*	promotes VG synthesis; inhibits choriogenesis?; inhibits oviposition? induces receptivity; inhibits brood protection?	promote choriogenesis, oviposition?	f
Diptera	Calliphoridae	*Phormia regina*	promotes sexual receptivity; not necessary for VG synthesis; promotes VG uptake	Promote VG synthesis	g
Diptera	Culicidae	*Aedes aegypti*	promotes pre-vitellogenic growth, competency for vitellogenesis, sexual receptivity	promote VG synthesis; deposition of vitelline membrane	h
Diptera	Drosophilidae	*Drosophila melanogaster*	promotes vitellogenesis and yolk uptake	promote pre-vitellogenic ovarian differentiation; promote yolk protein synthesis; inhibit vitellogenesis	i,j,k
Hemiptera	Lygaeidae	*Oncopeltus fasciatus*	no effect on VG-A synthesis; induces conversion of VG-A to mature form; promotes öocyte maturation	inhibit vitellogenesis?	l
Hemiptera	Reduviidae	*Rhodnius prolixus*	promotes but not *necessary* for VG synthesis and öocyte maturation; promotes VG uptake (patency)	inhibit vitellogenesis?; promote ovulation and oviposition	l
Hymenoptera	Apidae	*Apis mellifera*	not involved in maturation or later stages	probably not involved in adults	m,n

(Contd.)

Table 4 (Contd.)

Order	*Family*	*Species*	*Function of JH in female reproduction*	*Function of ecdysteroids in female reproduction*	*Ref.*
Hymenoptera	Apidae	*Bombus terrestris*	promotes VG synthesis and öocyte maturation	promote öocyte maturation	n
Hymenoptera	Apidae	*Melipona quadrifasciata*	not involved in maturation or later stages	probably not involved in adults	m
Hymenoptera	Formicidae	*Diacamma* (unnamed sp.)	not detectable in reproductive workers		n
Hymenoptera	Formicidae	*Lasius niger*	reduces egg output; apparently uninvolved in dominance interactions		o
Hymenoptera	Formicidae	*Solenopsis invicta*	promotes VG synthesis; higher levels promote VG uptake?; promotes egg output		p
Hymenoptera	Vespidae	*Pollistes dominulus*	promotes VG synthesis, öocyte maturation and reproductive dominance	promote reproductive dominance	n,p
Lepidoptera	Bombycidae	*Bombyx mori*	possibly none; certainly not required	declining titers promotes VG synthesis (and patency?)	q
Lepidoptera	Danaidae	*Danaus plexippus*	promotes VG synthesis, patency, choriogenesis	no effect on VG synthesis	q
Lepidoptera	Lasiocampidae	*Malacosoma pluviale*	suppresses VG synthesis?	promotes VG synthesis?	q,r
Lepidoptera	Lymantriidae	*Lymantria dispar*	suppresses VG synthesis?	promotes VG synthesis?	q,s
Lepidoptera	Noctuidae	*Helicoverpa zea*	promotes VG synthesis, patency, choriogenesis	none?	q

(Contd.)

Table 4 (Contd.)

Order	*Family*	*Species*	*Function of JH in female reproduction*	*Function of ecdysteroids in female reproduction*	*Ref.*
Lepidoptera	Noctuidae	*Heliothis virescens*	promotes VG synthesis, patency, choriogenesis	none?	q,t
Lepidoptera	Noctuidae	*Pseudaletia unipuncta*	promotes VG synthesis, patency, choriogenesis	none?	q
Lepidoptera	Nymphalidae	*Nymphalis antiopa*	promotes VG synthesis, patency, choriogenesis	no effect on VG synthesis	q
Lepidoptera	Nymphalidae	*Polygonia c-aureum*	promotes VG synthesis, patency, choriogenesis	no effect on VG synthesis	q
Lepidoptera	Nymphalidae	*Vanessa cardui*	promotes VG synthesis, patency, choriogenesis	no effect on VG synthesis	q
Lepidoptera	Pieridae	*Pieris brassicae*	promotes VG synthesis, patency, choriogenesis	no effect on VG synthesis	q
Lepidoptera	Pyralidae	*Diatraea grandiosella*	induces choriogenesis	declining titers promotes VG synthesis (and patency?)	q
Lepidoptera	Pyralidae	*Plodia interpunctella*		declining titers promotes VG synthesis	u
Lepidoptera	Saturniidae	*Hyalophora cecropia*	not necessary		q
Lepidoptera	Sphingidae	*Manduca sexta*	induces choriogenesis; increases the rate of (but not necessary for) VG synthesis; necessary only for late stages of vitellogenesis	VG synthesis initiated in absence of ecdysteroids; addition of ecdysteroids to pharate adults suppresses JH promotion of öocyte maturation	q,v,
Lepidoptera	Tortricidae	*Cydia pomonella*	induces choriogenesis, represses oviposition		r

(Contd.)

Table 4 (Contd.)

Order	*Family*	*Species*	*Function of JH in female reproduction*	*Function of ecdysteroids in female reproduction*	*Ref.*
Orthoptera	Acrididae	*Locusta migratoria*	promotes VG synthesis and öocyte maturation		w
Orthoptera	Gryllidae	*Acheta domesticus*	promotes VG synthesis and öocyte maturation	low levels promote maturation; high levels inhibit	x
Orthoptera	Gryllidae	*Gryllus bimaculatus*	promotes but not *necessary* for VG synthesis	more prominent involvement in maturation than JH?	x
Orthoptera	Gryllidae	*Gryllus campestris*	promotes but not *necessary* for VG synthesis	more prominent involvement in maturation than JH?	x
Orthoptera	Gryllidae	*Teleogryllus commodus*	promotes but not *necessary* for VG synthesis	more prominent involvement in maturation than JH?	x
Phasma todea	Heteronemiidae	*Carausius morosus*	unnecessary for VG synthesis; involved in VG uptake		y
Thysanura	Lepismatidae	*Thermobia domestica*	necessary for vitellogenesis	probably not involved	z

cases in which, for example, JH control has deviated from its canonical function in promoting vitellogenesis and öocyte maturation.

Lepidopteran (moths and butterflies) endocrinology has been particularly well studied (reviewed in Ramaswamy et al. 1997). Figure 4 is one fairly robust phylogenetic hypothesis for the Lepidoptera (based upon Kristensen and Skalski 1999) in which I have only included species for which vitellogenesis control mechanisms have been studied (not all such species are included). Next to the species names, I have indicated the stage at which mature öocytes (chorionated eggs ready to be laid) are produced, and if JH and/or ecdysteroids are involved in VG synthesis. Clearly, both reproductive features are highly variable. And while some of these character states show a strong phylogenetic signal (e.g. adult reproductive maturation among the butterflies—*P. brassicae*, *N. antiopa*, *D. plexippus*), others are most likely homoplasious (e.g. ecdysteroid regulation of vitellogenesis in the pyralid moth *P. interpunctella* and the silk moth *B. mori*). Interestingly, those taxa in which JH promotes vitellogenesis are also the only taxa among those considered that undergo öocyte maturation in the adult stage (the three butterflies included plus the tobacco budworm *H. virescens*). This finding raises the fascinating possibility that the reproductive control mechanisms are less associated with phylogeny than they are with selection on the timing of reproductive maturation (see below).

I have purposefully avoided attempting to map ancestral character states on the phylogeny in Figure 4. Since homoplasy would be extensive under any evolutionary scenario that one might favor, such an exercise would seem to be guesswork. Furthermore, as I mentioned previously, the taxon sampling here may not be completely random. It is intriguing, though, that the "typical" JH function in promoting VG synthesis is only found among the Macrolepidoptera, the most derived lepidopteran taxon, and specifically in only those macrolepidopterans in which öocyte maturation occurs entirely in the adult stage. Let us allow the assumption (and there is substantial evidence to support this assumption; see Ramaswamy et al. 1997) that the stage of öocyte maturation is intimately connected to the life history of the organism. The conclusion that follows from the apparent correlation between öocyte maturation stage and hormonal control mechanism is, therefore, that the hormonal mechanisms are coopted to follow suit with selection on insect life histories. Testing this hypothesis rigorously would necessitate more independent contrasts than presented in this limited phylogenetic data set. In any case, it seems clear that some caution is warranted in any attempts to reconstruct ancestral states for such characters, either in restricted insect taxa or among insects as a whole.

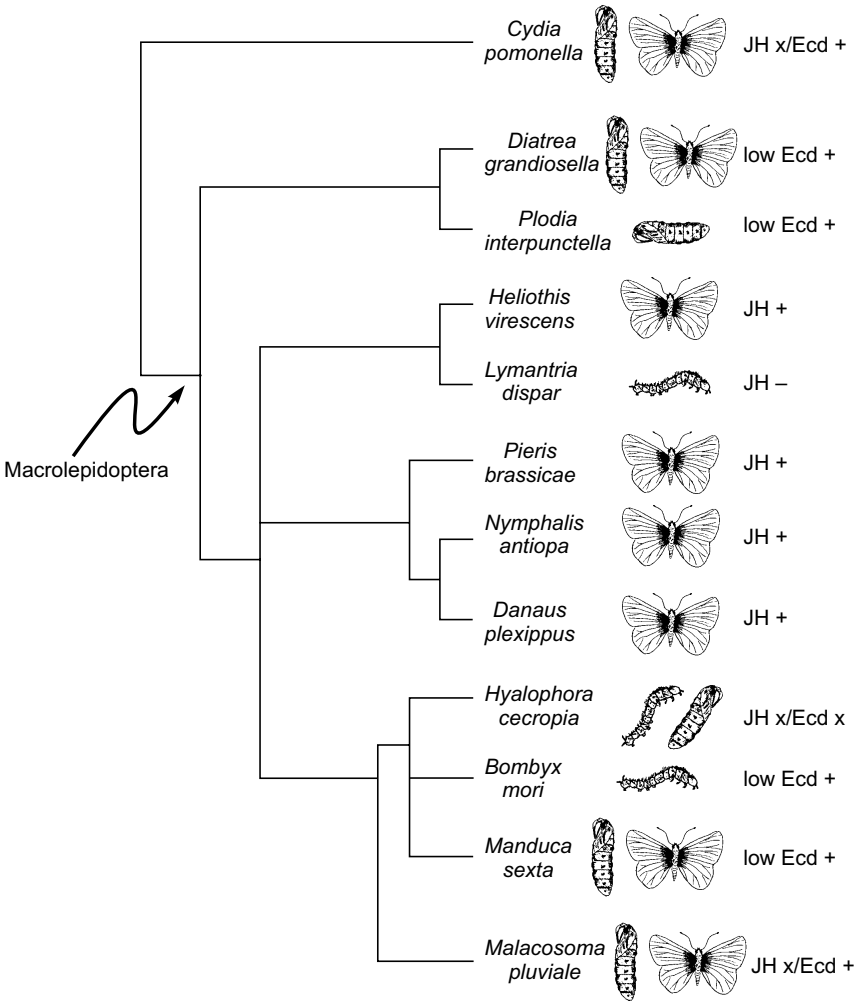

Fig. 4 Data from the Lepidoptera in Table 4 mapped onto a current phylogenetic hypothesis for the order (after Kristensen and Skalski 1999). Pictures indicate the stage at which öocyte maturation occurs, whether larval (e.g. *B. mori*), larval/pupal (*H. cecropia*), pupal (*P. interpunctella*), pupal/adult (e.g. *M. sexta*) or adult (e.g. *D. plexippus*). **+** indicates promotion of vitellogenesis by that hormone, **-** indicates repression, and **x** indicates that the hormone is apparently not involved in/required for vitellogenesis. See Table 4 for references.

As we will see in the next section, attempts to reconstruct ancestral character states have not only been popular in developing ideas regarding control mechanisms underlying the evolution of eusociality in insects, but,

indeed, seem to have led to some circular reasoning, where supposed similarities in control mechanisms are cited as evidence for close relationships among certain highly eusocial hymenopteran taxa.

Extreme Reproductive Plasticity: Queen-worker Dimorphism in Social Insects

Sociality has been hypothesized to have arisen numerous times independently: in termites, thrips (order Thysanoptera), aphids (order Hemiptera), beetles and multiple times in the Hymenoptera (ants, wasps and bees) (reviewed in Lin and Michener 1972; Halffter and Edmonds 1982; Stern and Foster 1996; Schwarz et al. 2007). While the close association between the evolution of sociality and a haplo-diploid mechanism of sex determination has long been cited (Hamilton 1964) as a critical correlate of sociality in the Hymenoptera (and the thrips as well), beetles and termites have the more typical diploid sex determination mechanism, and aphid sociality is related to clonality (reviewed in Stern and Foster 1996). These findings then beg the question of what factors have led to the evolution of sociality in insects in general, where certain members of a colony (queens, reproductive workers) have a higher reproductive potential than others (reviewed in Keller 1993; Reeve and Keller 2001). I will return to this point later. I will begin, however, with a discussion of some of the mechanisms underlying plasticity in öocyte maturation in the termites and a few groups of Hymenoptera. In the section on oviposition, I will revisit some of these same social insect groups, and consider some general questions regarding the evolution of eusociality in termites and hymenopterans.

Termites: Fixating on Nitrogen

As mentioned previously, hymenopterans and basal termites differ in at least one important respect: the termites have much more plasticity, as they can switch among reproductive and non-reproductive tasks depending on colony conditions.

I will return to a consideration of reproductive plasticity in the Termopsidae, the relatively basal family of termites that I introduced earlier. As is the case with ovariole number determination, termopsids are also quite plastic with respect to öocyte maturation. Secondary reproductives of *Zootermopsis angusticollis*, when raised with attendant workers, began laying eggs about 10 days earlier than unattended controls (Brent and

Traniello 2001a). Still, their egg laying rate, while enhanced in the presence of attendants, remained about 3-fold lower than that in primary reproductives under any condition. Interestingly, when the termites' diets (sawdust) included nitrogen (sawdust + uric acid) the secondaries achieved the same rate of egg laying as primaries, who did not show a change in rate with nitrogen supplementation (Brent and Traniello 2002).[16] Also, many termite species exhibit plasticity in cannibalism, consuming colony mates when nitrogen levels are low (Whitman et al. 1994). It seems, then, that nitrogen is limiting for secondary reproductives, and is a potent stimulator of öocyte maturation in termites. In sum, the dual ability of secondaries to increase their potential reproductive output by increasing ovariole number and maturation rate under certain conditions gives them the potential to attain primary reproductive-like levels of reproduction. As we shall see, this option, for several reasons, is not available to the majority of hymenopterans.

Ponerine Ants: The Workers Control the Means of Production (Sometimes)

Ponerines (Formicidae: Ponerinae) are a relatively basal lineage of ants, having less division of labor than most ants, and a class of workers (the so-called "gamergates") that can attain substantial levels of reproduction in the absence of their queen (reviewed in Peeters 1991). Some ponerine species lack queens altogether (see below). And unlike most hymenopteran workers, ponerine gamergates can mate with males. This is key, because it allows them the potential to raise female daughters, both workers and queens.

In the ponerine ant *Gnamptogenys menadensis*, worker-run colonies are the norm: Gobin and colleagues (1998) observed only two queenright colonies out of 37 examined in Sulawesi. There are several gamergates (between 1 and 14; mean=5) in each of the worker-run colonies. When all of the gamergates are removed, a subset of the virgin workers ("dominants") begin dominance interactions, involving agonistic behaviors (antennal boxing and biting; Gobin et al. 2001). Non-reproductive workers ("subordinates") did not engage in such behaviors, but occasionally ganged up on individual dominant worker, inevitably converting such a worker to a subordinate. After several weeks or months, some of the dominants began sexual "calling" behavior (i.e. adopting a pheromone-releasing posture), and from that time forward, dominance interactions were no longer observed.

[16] Nitrogen contents were determined in all samples by an elemental analysis assay sensitive to within 0.003%.