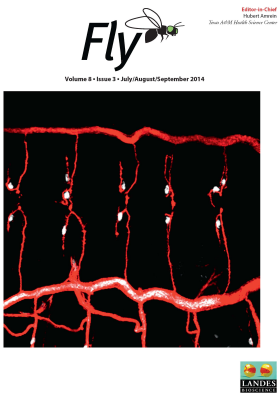

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REVIEW

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The song of the old mother: Reproductive senescence in female drosophila

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Keywords: fecundity, fertility, maternal condition, reproductive senescence, trade-offs

Among animals with multiple reproductive episodes, changes in adult condition over time can have profound effects on lifetime reproductive fitness and offspring performance. The changes in condition associated with senescence can be particularly acute for females who support reproductive processes from oogenesis through fertilization. The pomace fly *Drosophila melanogaster* is a well-established model system for exploring the physiology of reproduction and senescence. In this review, we describe how increasing maternal age in Drosophila affects reproductive fitness and offspring performance as well as the genetic foundation of these effects. Describing the processes underlying female reproductive senescence helps us understand diverse phenomena including population demographics, condition-dependent selection, sexual conflict, and transgenerational effects of maternal condition on offspring fitness. Understanding the genetic basis of reproductive senescence clarifies the nature of life-history trade-offs as well as potential ways to augment and/or limit female fertility in a variety of organisms.

"While I must work because I am old,
And the seed of the fire gets feeble and cold."
Song of the Old Mother
—William Butler Yeats

Introduction

Fertility is a major determinant of an animal's inclusive fitness. In some species, individuals experience a single reproductive episode followed by death (semelparity), while in other species individuals engage in multiple reproductive episodes during their lives (iteroparity). Among iteroparous animals, senescence can have large effects on female reproductive physiology and subsequent fitness. Senescence is characterized as decreased physiological function over time caused by intrinsic and stochastic changes at system, organ, tissue, cell, and genetic levels.[1,2] Female reproduction is an interesting system with which to explore the process and consequences of senescence, because high and sustained fertility relies on multiple regulated processes including oogenesis, ovulation, sperm storage, fertilization, gestation (in some organisms), and oviposition/birth. While age-related effects on reproductive performance are hypothetically possible in semelparous species, evidence for their existence is limited.[3] In contrast, reproductive senescence has been observed in natural populations of iteroparous animals from diverse taxonomic groups including birds, mammals, reptiles, and insects (reviewed in[3]). It is also a well-documented phenomenon in humans in which fertility declines dramatically during a woman's fourth decade of life (reviewed in[4]). The iteroparous pomace fly, *Drosophila melanogaster*, is a well-established model system for studying the processes and regulation of both reproduction and senescence.[2,5-7] However, many laboratory studies characterizing female reproductive physiology or testing hypotheses related to sexual selection focus only on young females in their reproductive prime (e.g.,[8-13]) and/or allow both male and female age to increase concurrently.[14-16] Since male reproductive physiology also changes with increasing age,[17,18] controlling for male age in experiments helps distinguish the effects of female age, male age, and their interactions, on phenomena associated with reproductive senescence.

A better understanding of the nature of female reproductive senescence can identify common mechanisms underlying apparently different phenotypes associated with senescence, improve our understanding of male-female dynamics required for successful reproduction, and highlight potential approaches for regulating fertility in agriculture and conservation settings. Additionally, it clarifies the nature and circumstances of trade-off theory and its genetic underpinnings, which suggests that the female's allocation of limited resources toward reproductive fitness or somatic maintenance confers a cost for the other.[1,19] Here, we review current knowledge about the effects of female age on various aspects of reproduction. We focus on the physiological processes involved in mating behavior, oogenesis, sperm fate, and offspring viability in *D. melanogaster* (summarized in **Table 1**), with the inclusion of information about other Drosophila species as available, and on the genetic foundation of reproductive senescence. We also identify where experimental research has distinguished female age effects from parental (both

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Table 1. Reproductive morphology, physiology and behaviors affected by increasing female age in *Drosophila melanogaster*

Characteristic	Change with ↑ female age	Female fly strains	Male age controlled?	Female age range (days post-eclosion)	References
Pre-mating					
Female attractiveness to males					
intact	no change	CS	Y	5–20	37
decapitated	decline	Novosibirsk; CS, Fv	Y; Y	1–20; 8 and 52	36; 39
Virgin latency to mating	increase	Dahomey; CS	Y; Y	1–40; 5–20	27; 37
Virgin female receptivity	decline	Dahomey; Pacific; CS	Y; U; Y	1–40; 1–42; 5–20	27; 35; 37
Female latency to remating	no change	Dahomey	Y	1–40	27
Female remating	decline	Dahomey	Y	1–40	27
CHC profile	increased long-chain compounds	CS, Fv	Y	7–65	39
Peri- and Post-Mating					
Copulation duration 1st mating	increase at intermediate ages (8–19 d pe)	Dahomey	Y	1–40	27
Copulation duration re-mating	decline; none	Dahomey; LHm	Y; Y	1–40; 6 and 18	27; 46
Sperm transfer	decline	LHm	Y	6 and 18	46
Sperm in SR	no change	LHm	Y	6 and 18	46
Sperm viability in SR	no change	Macon, GA	Y	8 and 31	55
Last male paternity success	decrease	inbred lines 79L, 67L, 58S	Y	3–31	56
Fecundity					
# Ovarioles	no change	w^{1118}	Y	0–43	59
GCS number	decline	CS, w^{1118}; yw; yw*	Y; N; U	3–40; 7 and 63; 3–36	59; 62; 66
GCS & SSC proliferation	decline	w^{1118}; yw	Y; N	3–40; 7 and 63	59; 62
Oogenesis	decline	field-collected from Austria, South Africa, and Zambia; B, O	N*; N	entire adult life; 4–64	15; 65
Oocyte morphology	small yolk sacs, ventralized dorsal appendages; smaller size + increased variability in size	w^{1118} + CS; OR	Y; N	1–37; 1–22	59; 74
Oocyte quality	increased quality of carbonylated proteins; changing proportions of mtDNA variants	w^{1118}; field-collected from Brownsville, TX	Y; N	5 and 35; 2 and 14	77; 78
# Eggs laid	decline	F_1 of vestigial x Champetieres; field-collected from Austria, South Africa, and Zambia; Dahomey; U; CS, w^{1118}; CO derived from IVES; CO derived from IVES	N; N*; Y; Y; Y; N; N	0–50; entire adult life; 1–40; 0–48; 0–48; 12d from egg-death; 12d from egg - death	14; 15; 27; 58; 59; 61; 60
SP-effect on fecundity	lost	Dahomey	Y	1–40	27
Fertility					
Egg-adult viability	decline	Dahomey; Dahomey; CO & ACO derived from IVES; HAM/LAM derived from Basel, Switzerland strain	N; Y; N; N	1–15; 3 and 28; 22–66 d from egg; 15–60 d from egg	16; 27; 60; 81
Embryonic viability	decline	F_1 of vestigial x Champetieres; field-collected Austria, South Africa, and Zambia; Dahomey; CS, w^{1118}; OR; HAM/LAM derived from Basel, Switzerland stock	N; N*; N; Y; N; N	1–60; entire adult life; 1 and 15; 0–37; 1–22; 15–60 from egg	14; 15; 16; 59; 74; 81
Larvae-pupa	increase	Dahomey	N	1 and 15	16
Larval-adult viability	decline	HAM/LAM derived from Basel, Switzerland strain	N	15–60 d from egg	81
Pupal-adult	increase	Dahomey	N	1 and 15	16
Preadult development rate	decline; no change	wt/CyL2 flies - wt from Kosovo, Yg strain; OR	N; N	5 and 25; 0-final 24h of fertility	82; 84
Offspring performance					
Longevity	decreased	CS, UGA98, inbred strains 79L, 67L, 58S, 35S; OR; U	Y; N; N	7–63; 0- final 24h of fertility; 3–18	83; 84; 85
Phenotypic asymmetry	increased	OR	N	1–22 d pe	74

Abbreviations: CHC, cuticular hydrocarbons; GSC, germline stem cells; SCS, somatic stem cells; CS, Canton-S; OR, Oregon-R; yw*, results also found in several other wild-type lines; T. Xie and A. Spradling, pers. comm.; Y, male age controlled within a narrow age-range during experiments; N, male age allowed to increase with increasing female age; N*, males replaced every 3 weeks; U, male age or strain not provided.

male and female) age effects, identify gaps in our understanding of reproductive senescence, and suggest avenues for future research. For additional comprehensive reviews on Drosophila senescence and aspects of reproduction including, courtship, oogenesis, responses to male mating stimuli, sperm storage and sperm precedence, please refer to refs.[2,5-7,20-25]

Female Reproductive Senescence

Overview

Female *Drosophila melanogaster* are fertile within 24 h of eclosion. Fertility peaks within the first week of adulthood then steadily declines with age until females become infertile ([26,27], **Table 1** and reviewed below) or die. Female lifespan is affected by genetic (nuclear and mitochondrial) background,[28,29] husbandry practices allowing overlapping generations versus those enforcing discrete generations and high early fecundity,[26] adult caloric and dietary restriction[28,30-32] and mating frequency.[33] In bottle and cage cultures, females live approximately 20–44 d post-eclosion with regular exposure to males[26,33,34] and nearly twice as long when unmated[26,34] (MBQ personal obs).

Courtship and Mating

Courtship is a remarkably complex behavior displayed by Drosophila species, and particularly in the extensively studied species *Drosophila melanogaster* (reviewed in[20]). It involves the exchange of visual, chemical, and auditory cues between males and females. Studies of courtship tend to focus more on the male, the more obvious active party, yet the female's acceptance ultimately determines the male's mating success.[35,36] Age affects female attractiveness to males, as well as female behaviors associated with courtship, receptivity, and mating success[35,37] and reviewed in.[38]

While male courtship vigor toward active (intact) females has not been observed to change with increasing female age (5 d–20 d post-eclosion, hereafter abbreviated as d pe;[37]), male discrimination based on female age is detected under conditions where some female stimuli are isolated. When females are immobilized and decapitated to prevent rejection responses, males spend ~50% less time courting old females than young females (female ages 20 d–1 d pe,[36]; 52 d vs 8 d pe,[39]). However, female movement is an important visual signal for mate recognition and courtship initiation. Males perform less frequent and shorter courtship bouts when the females are completely inactive as compared to when they are active.[40] Several studies have described age-related declines in locomotor activities such as geotaxis, phototaxis, exploratory activity (movement from original position), and spontaneous activity (general movement) that, while not directly related to courtship, could decrease female exposure to males as well as movement cues needed to initiate male courtship.[41-43] Direct effects of age-related changes of female movement on mate recognition and courtship await examination.

Females exude a pheromonal bouquet consisting of cuticular hydrocarbons (CHCs) derived from fatty acids. CHCs play major roles in mate identification, evaluation, and communication.[20,44] Recent technical advances have revealed greater complexity in CHC bouquets than previously appreciated as well as changes in CHC composition and relative amounts.[45] For example, CHC profiles changed in consistent ways most notably by producing higher proportions of long-chain compounds with increasing age in females from 2 different fly strains.[39] A young female CHC profile was both necessary and sufficient for males to discriminate between chronologically young and old females; male courtship preferences for young vs. old females corresponded with the female CHC profiles, disappeared when CHCs were removed from young and old females by hexane washing, and could be re-directed toward older females when coated in CHCs from younger females (8 d pe vs 52 d pe;[39]).

Female receptivity peaks at >90% 1–2 d pe, then begins to decline approximately 7 d pe in virgin females[27,35,37] and as early as 5 d pe in previously-mated females.[27] By 3 weeks post-eclosion, receptivity declines substantially to between 50% and 75% of maximal levels in previously virgin flies[27,35,37]) and ~20% of maximal levels in previously-mated females.[27] The decline in mating success is due to both increased female latency to mating and an increased proportion of unreceptive females. Virgin female latency to mate with wild-type males doubled in duration from <20 min when ≤8 d pe to >50 min after 26 d pe ([27] also see[37]). However, this was not observed in females remating repeatedly whose mating latency remained unchanged with age at ~20 min.[27] As females age, they become progressively less receptive to males so by 20 d pe, females are 3-fold less receptive than they were at 5 d pe ([37] also see[35]). Female receptivity in general corresponds with, but is not directly responsive to, ovarian development, which increases rapidly ≥2 d pe, then declines >6 d pe. A potential common regulating factor is juvenile hormone (JH) signaling which, when augmented in females, increases female receptivity relative to controls.[35]

Copulation duration may also be affected by increasing female age. Among virgin females, females of intermediate ages (8 d–19 d pe) had longer copulation durations (~18.5 min) than younger (1 d–7 d pe; ~17 min) and older (22 d–40 d pe; ~16.5 min) females. However, when females had previously mated with wild-type males, as is more likely to be the situation in natural populations, older females (≥22 d pe) had shorter copulation durations (<11 min) than earlier ages (13 min–19 min).[27] Another study failed to detect a difference in copulation duration between previously-mated young (6 d pe) and old (18 d pe) females possibly because the females had not aged to the extent to express this response[46] (although this study detected other female age-related reproductive effects, reviewed below).

Changes in the female nervous system are implicated in the loss of female sexual receptivity, although examination of the direct relationships between nervous system function and reproductive behaviors are still needed. First, age related changes in fly responses to repellent olfactory cues[42] suggests that females' abilities to detect, integrate, or respond to mating-related sensory cues become compromised over time. Second, the observed decrease in sexual receptivity in older females resembles increased mating avoidance behavior observed in dopamine-depleted

females,[37] indicating possible dopaminergic modulation of female sexual receptivity. Additionally, a decrease in whole body levels of dopamine >7 d pe and degeneration of catecholaminergic cell bodies in the thoracic ganglia and antennal glomeruli is consistent with neurodegeneration in aging flies.[37] Together, these results suggest that functional senescence in the nervous system may contribute to age-related changes in female sexual behaviors, although this hypothesis has not been examined directly.

Extending the examination of female reproductive senescence to non-melanogaster Drosophilids highlights conserved aspects of the aging process as well as shows how aging can occur differently – perhaps as a result of unique developmental constraints or trade-offs. Despite high initial receptivity, virgin *D. simulans* female receptivity begins to decline by 6 d pe. Like some observations of *D. melanogaster*, this decline in receptivity appears to be due to increased percent of unreceptive females rather than an increased latency to mating ([35], but see[27]). Unlike *D. melanogaster* and *D. similans*, intermediate-aged females (17–18 d pe) of the sibling species *Drosophila ananassae* (mean lifespan = 90 d pe[47]) and *D. bipectinata* (mean lifespan = 60 d pe[48]) appears to be the most reproductively-active age cohort exhibiting the shortest and most vigorous courtships as well as the highest mating success ([48,49], respectively). Like *D. melanogaster*, mating behaviors in both species declined beyond this "middle age," but differed in several aspects of mating behavior including male courtship, female rejection, and latency to mate. Copulation duration changed little between old (32–33 d pe) and young (2–3 d pe) females.[48,49] Finally, 2 other species, *D. gaucha* and *D. pavani*, had slightly different responses with increasing female age; female latency to mating was longer in old versus young *D. gaucha* females, while copulation duration was longer in older vs. younger *D. pavani* females.[50] However, the biological significance of these age-related effects are difficult to interpret due to the young ages tested relative to their typical lifespans (old females = 18 d–20 d pe, young females = 10 d pe, lifespan = 100–300 d pe). So while female mating behaviors in other Drosophilids generally decline with increasing age, the timing of the decline and behavioral manifestations of the decline vary. The basis of these differences is unknown and could be due to species-level differences in investment in reproductive function, developmental constraints, or different assay conditions. These cases highlight the diversity in phenotypes associated with female reproductive senescence and the need to relate these differences to differences in physiology and environmental constraints.

Ejaculate transfer, storage, and competition

In *D. melanogaster*, female age continues to affect reproductive success peri- and post-copulation. During mating, males transfer a complex ejaculate composed of sperm, proteins (called seminal fluid proteins, SFPs), peptides, and sugars.[6,51,52] Upon transfer to the female, these compounds interact with each other, female tissues, and ejaculates of previous mating males, if present. Males transfer 15% fewer sperm to older (18 d pe) females than younger (6 d pe) females.[46] While it is unknown whether males or females limit ejaculate size and/or composition, strategic ejaculate transfer by males exists under other circumstances[46,53,54] suggesting that males may be capable of modulating ejaculate components in response to female condition.

Once transferred, the ejaculate components reside within discrete regions of the female's body. Sperm are retained and maintained in storage organs of the reproductive tract: the seminal receptacle and paired spermathecae. SFPs localize, in some cases by association with sperm, to a variety of structures and regions such as the female storage organs and central nervous system where they influence reproductive function for various periods of time after mating (reviewed in[5]). Although female age influences the quantity of sperm transferred by the male, it does not appear to affect the numbers of sperm stored in the seminal receptacle[46] or their viability between young (6 and 8 d pe, respectively) and old (18 and 31 d pe, respectively) females.[55]

After mating, females generally remain unreceptive to courting males for 3–4 d (reviewed in[5]). Receptivity often resumes before all of the sperm stored from previous matings is depleted and female remating creates a situation where sperm from different males are competing for access to ova (sperm competition) and females can influence paternity of those ova (female sperm preference or cryptic female choice). Among young females, second male paternity success is approximately 80%. However, this precedence declines significantly (~10% of the untransformed values) as females age (3.5 to ≥17d pe,[56]). The cause of this decline remains unexplored, but several recent findings suggest potential mechanisms. Upon remating, sperm from the first mating male are released from female storage sites where they interact with the newly-received sperm in the bursa copulatrix (also called uterus) before the sperm mixture is (re-)stored.[11] With males transferring fewer sperm to older females,[46] there may be relatively lower representation of second male sperm in the newly-established fertilization set, from which resident sperm would be drawn to fertilize the ova. Alternatively, decreased fecundity of older females (see next section), may result in decreased depletion of stored sperm from the first mating male, which also increases his relative representation in the subsequent fertilization set. Finally, accelerated ejection of the bursal sperm mass by older females could alter storage dynamics in a manner that would be disadvantageous to second-mating males. A more complete and detailed description of sperm storage dynamics with increasing female age will help elucidate these possibilities.

Fecundity

A common marker of reproductive success in *D. melanogaster* is fecundity, which is experimentally measured as the number of eggs laid (e.g.,[14,27,48,49,57]). While a female's capacity to produce eggs is not the only determinant of reproductive success, it is critical, because it sets an upper-limit to fertility. With regular exposure to males, daily fecundity changes with age. It rapidly increases peaking at >60 eggs/day (range of 65–104 eggs/day in different genetic backgrounds) around 4–7 d pe followed by a progressive decline with fecundity levels half of peak levels 22 d–28 d pe and resulting in either infertility by 50 d pe[14,15,27,58,59] or a fecundity plateau.[60,61] Changes in fecundity over time have recently been modeled with more precision using

3 different recently wild-caught populations. In these populations, a steady decline in fecundity was observed from 4 d–35 d pe followed by an exponential decline in fertility.[15] Genetic variation among populations was also documented for the duration of some reproductive phases including the time to peak fertility, and the timing and rate of the exponential decline in fertility, but not the rate of gradual decline in fertility.[15] The mechanisms underlying this fecundity decline include age-related changes in oogenesis and responses to the male seminal protein sex peptide (SP). Additional mechanisms are likely to be involved, but are currently unknown.

Oogenesis is the cellular basis of fecundity and, under suitable temperatures and nutrient availability, is continuous throughout most of the female's adult life. In *D. melanogaster*, each of the 2 ovaries is composed of 10–20 tubular ovarioles. The distal end of each ovariole, the germarium, contains a limited number of germline and somatic stem cells (GSC and SSC, respectively), each within their own stem cell niche.[59,62] As oogenesis begins, select GSC progeny differentiate becoming cystoblasts. Each cytoblast undergoes 4 mitotic divisions producing the germline cyst; 16 cytoplasmically-connected cells of which 15 are nurse cells and one is an oocyte. Surrounded by SSC-derived follicle cells, this unit becomes the egg chamber. The egg chamber leaves the germarium and moves down (proximally) the ovariole while undergoing vitellogenesis and further differentiation.[22,63,64] Oogenesis is subdivided into 14 distinct morphological stages, grouped into 2 major phases: previtellogenic (stages 2–6) and vitellogenic (stages 8–14) phases.[22,63] Both phases can be adversely affected by increasing female age. In a study of females derived from a recently field-caught population, ovaries from aging or newly dead females contained a limited number of mature oocytes, but no other vitellogenic oocytes beyond the early stages 8/9 ([15], although the increasing age of experimental males potentially impacts this result). Another study using a laboratory adapted population and allowing both mating males and females to age found that female age primarily affected the previtellogenic
phase[65] with an increased proportion of ovarioles lacking previtellogenic oocytes,[65] but did not find evidence that vitellogenic activities changed with increasing female age.

The decline in oogenesis with increasing female age has several, not mutually exclusive, potential causes. Decreased previtellogenic activities in older females might be attributable to the loss of GSCs and accompanying changes to the ovarian stem cell niche.[62] As females age, they have fewer germline stem cells.[59,62,66] However, this reduction is not sufficient to explain the dramatic reduction in fecundity over this span of time. Reduced fecundity appears to be largely due to reduction in stem cell proliferation ([62] as much as 3-fold[59]) up to ~25 d pe complemented by a dramatic increase in apoptotic cell death in cystoblasts, germline cysts, and egg chambers >25 d pe.[59] Proliferative capacity is influenced by several factors in the niche and GSCs themselves. Within the niche, levels of BMP signaling, necessary for cell renewal, and E-cadherin, a cell-adhesion molecule, decrease with increasing female age.[62] Increased BMP signaling in either the niche or GSCs augments GSC proliferation. Likewise, increasing SOD, an enzyme decreasing oxygen free-radicals, promotes GSC proliferation and longevity.[62] Declining vitellogenic activity as females age might be caused by changing responsiveness to circulating hormones. Both JH and ecdysone are involved in vitellogenesis by promoting yolk protein accumulation in developing oocytes as well as its synthesis in the insect fat body.[67-69] Because reduced levels of JH or ecdysone result in extended adult longevity,[70,71] the age-related decline in vitellogenic activity seems less likely attributed to declining hormone titers and more likely due to declining JH and/or ecdysone receptor abundance or sensitivity in the ovary or fat body, respectively. Finally, another factor affecting the decline in fecundity appears to include age-related changes in female response to the male SFP Sex Peptide (SP). SP induces multiple physiological and behavioral responses in females including increased egg laying and reduced receptivity to males.[72,73] Some of these responses are age-dependent; while receptivity decreases in females of all ages, only young previously-virgin females increase fecundity.[27] The ability of females to detect or respond to SP signals has been hypothesized to diminish with age. Senescence-related changes in female responses to other male seminal fluid proteins, some of which affect ovulation and egg laying, remains unknown.

Egg morphology is determined during oogenesis and corresponds with subsequent viability. Egg length is positively correlated with embryonic viability and developmental rate ([74] and reviewed in[75]). Increasing parental age (1 d–22 d pe) correspond with 1.2% decreased mean egg length and increased variability in egg length,[74] the latter reflecting increasing developmental instability, as well as decreased viability.[74,76] There is also an increase in morphological abnormalities such as small yolk sacs and ventralized dorsal appendages from being nearly absent in young females (≤5 d pe) to >20% incidence in females 32 d pe.[59] Changes in egg quality also matter; eggs of older females (35 d pe) contain higher levels of carbonylated proteins than young females (5 d pe). Increased carbonylation reflect decreased control over protein quality and corresponds with decreased viability, reflecting declining maternal protein quality homeostasis.[77] Finally, offspring of older parents (11 d–22 d pe) have higher proportions of a mtDNA variant than offspring of young parents (0 d–3 d pe).[78] While this does not appear to reduce embryonic/offspring quality itself, it provides additional evidence that increasing parental age results in changes in oocyte quality – some of which are likely deleterious.

In non-melanogaster species of Drosophila, fecundity exhibits a recurring pattern of a peak relatively early in adulthood, then a progressive decline. In both *D. virilis* and *D. pseudoobscura*, fecundity peaks ~2 weeks post eclosion, followed by a decline throughout the remainder of their lives ([57,79], respectively). Compared with the pattern seen in *D. melanogaster* with a peak around 4 d pe, non-melanogaster species appear to reach their reproductive prime at a later point in life. Both *D. ananassae* and *D. bipectinata* females exhibit the highest receptivities to mate, courtship activities, and fecundities at slightly older ages (17–18 d pe;[48,49]). A continuous decline in fecundity from 3 d–33 d pe was observed using aging males and females.[47] This pattern of fecundity corresponds to, and may be controlled by, the number

of ovarioles which are highest during middle age (n > 25 ovarioles per ovary) and lowest in old females (n = 11–13 ovarioles per ovary).[48,49] If reproductive organ development in these species resembles that of *D. melanogaster* and is largely complete by eclosion,[64] then it is not clear how the difference in ovariole number with age might arise. Ovarioles may become active and later be resorbed at different ages. Alternatively, differential death among age cohorts could affect final ovariole number. Changes in mating success also appear positively associated with changes in fecundity, suggesting possible interaction between these 2 components of female reproduction. Perhaps this reflects a particularly high investment required to produce oocytes, which set an upper limit to fecundity - similar to observations of spermatogenesis in several species of Drosophila.[80]

Fertility

Generally measured as total adult offspring, fertility decreases with increasing maternal age ([16,27,60,81], although only[27] controls for male age). In the later study, young (3 d pe) females produced, on average, >250 offspring over 6 d while older females (28 d pe) produced >60% fewer offspring over the same duration.[27] While some of this is attributable to decreased fecundity (reviewed in previous section), it is also due to decreased offspring viability. Measured as survival from egg to adult, offspring viability is initially high (>70%) for young (3 d pe) females then steadily declines over the following 37 d pe.[27] A similar trend is observed as parental age increases: offspring of old (15 d pe) parents had ~3% lower viability than offspring of young (1 d pe) parents,[16] and additional studies found a negative relationship between parental age and offspring viability.[60,81] One study failed to detect a difference in offspring viability between old parents (males and females >25 d pe) and young parents (>5 d pe), although the offspring of old parents developed significantly (16.4%) more rapidly than young parents.[82]

Fertility is studied more precisely by observing the effects of female age on offspring viability at different life history stages including embryonic, larval, pupal, and adult. The degree to which maternal age affects these life stages varies.[16] Embryonic viability (often measured as percentage of eggs hatching) is high (>80%) when females are <7 d pe and decreases by 9–65% as females age (>23 d pe) ([14-16,59,74,81], although only[59] controls for male age). This developmental stage best predicts overall offspring viability.[81] However, failure of eggs to hatch can be due to fertilization failure and/or embryonic death. The relative contributions of these 2 factors have not been reported. While larval- and adult-stage viabilities appear to be affected by increasing parental age, the nature of these effects has not been examined as extensively as has embryonic viability and effects of male and female age have not yet been distinguished. Larval-adult viability decreases with increasing parental age, but to a smaller amount (10%) than embryonic viability.[81] Surprisingly, larval - pupal viability is positively affected by increasing parental age, although the benefit was relatively small (3.4%,[16]).

Finally, the effects of maternal age at reproduction on offspring fitness can be transgenerational and varied. In 5 of 6 experimental lines (2 outbred and 4 inbred lines), increasing maternal age corresponded with decreased lifespan of daughters.[83] Additional experiments also found that increasing parental age (both male and female) corresponded with decreased adult offspring longevity.[84,85] Older females also have offspring with decreased developmental stability, as reflected in increased asymmetry in sterno-pleural chaeta number, which corresponds with decreased offspring viability and longevity.[74]

Non-melanogaster species of Drosophila also show fitness costs for increasing maternal age. Offspring viability decreases with increasing maternal age in *D. serrata, D. paulistorum, D. pseudoobscura*, *D. persimilis*, and in hybrids of the latter 2 species.[86-88] Like *D. melanogaster*, maternal age corresponds with decreased offspring developmental stability (measured as phenotypic variance in wing size) in a parthenogenetic strain of *D. mercatorum*.[89] Furthermore, the results across 2 generations were cumulative, as offspring of old mothers and old grandmothers had the highest levels of developmental instability.[89] Whether or not the observed developmental instability contributes to decreased offspring viability or is a corresponding indicator of decreased viability is unknown. Cumulative, multigenerational effects of maternal age are also observed in *D. serrata*, in which offspring of young mothers and young grandmothers had higher viability than any other maternal/grandmaternal age combination.[86] The mechanism of the cumulative negative effects of maternal age on offspring development and performance are unknown and could include genetic, epigenetic, or maternal effects such as oocyte provisioning.

The Genetic Basis and Architecture of Female Reproductive Senescence

Female reproduction in lines selected for long or short life

Increasing female age corresponds with declines among multiple facets of reproductive physiology and, ultimately, fitness in *D. melanogaster*. While not mutually exclusive, the decline could be due to the accumulation of damage over time or a trade-off with high early fertility.[19,33,90-94] An underlying genetic contribution to reproductive senescence is supported by the observations that *D. melanogaster* lines can be selected for early and late life fertility (reviewed below) and that the identity and transcriptional activities of genes related to reproduction changes with age.[95,96] Hypotheses offering genetic explanations for damage accumulation and trade-offs are mutation accumulation[90,91] and antagonistic pleiotropy,[92] respectively. The use of different genetic backgrounds, including inbred lines, and selection experiments has been valuable for providing information about the genetic foundation of the aging process, as well as describing and distinguishing between these 2 possibilities (reviewed in[97]). The general approaches to selection experiments have included: 1) allowing females to age for varying periods of time before collecting embryos for the next generation (young = 1–8 d pe, old = ~16–30 d pe, and occasionally intermediate ages,[26,97,98] and 2) subjecting populations to different levels of extrinsic adult mortality.[81,99]

Experimental evidence supports the role(s) of mutation accumulation in reproductive senescence. Evidence for this consists of a documented increase of additive genetic variation with age. Using this approach, a small number of studies examining female fecundity over time offer direct evidence for mutation accumulation ([100,101], but see[102]), although these studies did not control for effects of increasing male age. For example, one study documented more than two-fold higher levels of additive genetic variation in older (28–31d pe) parents relative to younger (3–14 d pe) parents,[101] reflecting the increased prevalence of alleles with deleterious, late-life effects.[103,104] In other cases, a role for mutation accumulation has been inferred by the failure to document antagonistic pleiotropy.[105,106] However, lab stock cultures maintained with short (2 week) generation times may permit late-acting mutations with deleterious effects to accumulate, thereby resulting in environmental-induced evidence for mutation accumulation.[26,107,108]

Multiple studies designed to detect a role for antagonistic pleiotropy on reproductive senescence have generated various levels of support for its existence (reviewed in[106]). Evidence for antagonistic pleiotropy consists of documenting a negative genetic correlation between longevity and early fecundity (or other early-late life measures of fertility) (described in[101]). Using this approach, several studies have provided strong support for the existence of antagonistic pleiotropy evidenced by an unequivocal, statistically significant relationship between early age and late age fitness components.[60,61,97,98,109] For example, subjecting lines adapted to later-life reproduction (and longer life) to selection for earlier reproduction resulted in corresponding acceleration of low fertility plateaus compared with unselected lines.[61] Other studies generated results that were consistent with the presence of antagonistic pleiotropy but provided less strong support for the presence of antagonistic pleiotropy due to the presence of trends that were not statistically significant (for selected females[102]; depending on the method of analysis[81]) or the presence of negative correlations between early and late life history traits were balanced by the presence of positive correlations.[101] The presence of balancing positive correlations indicates that while antagonistic pleiotropy may contribute to female reproductive senescence, it is not likely to be the only cause of it. Finally, some studies have failed to detect the presence of antagonistic pleiotropy - even when measuring multiple facets of lifetime fertility.[100,105,106] These studies suggest that either mutation accumulation is the primary cause of reproductive senescence or that the particular measure(s) of fertility selected are not those involved in the negative genetic correlation with late life fitness.[105] Studies supporting the existence of antagonistic pleiotropy have also encountered challenges regarding gene-environment interactions and unintended selection for reproductive traits.[26,97] In summary, there appears to be evidence in support of both mutation accumulation and antagonistic pleiotropy in female reproductive senescence. However, inconsistently controlling for gene-environment interactions has made it difficult to ascertain the relative contributions of these 2 phenomena to reproductive senescence.

Female reproduction in longevity mutants

In *D. melanogaster*, single gene mutations extending lifespan, some of which are evolutionarily conserved,[110,111] have led to important discoveries concerning the processes involved in senescence. These processes include stress resistance, dietary restriction, and insulin/IGF signaling.[25,112] As described in this review, female senescence typically results in decreased reproductive productivity. If mutants with extended lifespan also have extended, and therefore increased, lifetime fertility, then aging and reproduction are not inextricably connected genetically or physiologically. While several mutations conferring extended lifespan exhibit decreased reproductive output, suggesting the existence of negative (antagonistic) pleiotropic effects between longevity and reproduction, the agents mediating the potential trade-offs are not well understood ([113,114], reviewed in **Table 2**). Other mutations that affect lifespan but not fertility, and experiments carefully controlling the location and timing of gene expression suggest that longevity and fertility may be physiologically separated ([115,116], reviewed in **Table 2**). Like selection experiments,

Table 2. Genes implicated in both female longevity and reproduction in *Drosophila melanogaster*

Gene	Ontology[1]	Normal effect on longevity	Normal effect on reproduction	References
Heat shock protein 70 (Hsp70) (CG31366)	Response to heat	↑	↓ embryonic viability; no effect on fecundity	125
Methuselah (mth) (CG6936)	G-protein coupled receptor	↓	no effect on fecundity; ↑ fertility	116; 118
I'm not dead yet (Indy) (CG3979)	Citrate and succinate transporter membrane activity	↓	no effect on fecundity; ↓ fertility	119; 137
Insulin-like receptor (InR) (CG18402)	Insulin-activated receptor	↓	↑ vitellogenesis	138
Chico (CG5686)	Insulin-receptor binding	↓	↑ vitellogenesis	111; 141; 142; 143
Insulin-like peptides ILP2, ILP3, ILP5 (CG8167, CG 14167, CG32051)	Insulin-receptor binding	↓	↑ fecundity; ↑ remating	147; 148
Hebe (CG1623)	none	↑	↑ fecundity	149
Magu (CG2264)	heparan sulfate proteoglycan binding	↑	↑ fecundity	149

Symbols: 1. Information from: St. Pierre SE, Ponting L, Stefancsik R, McQuilton P, and the FlyBase Consortium (2014). FlyBase 102 - advanced approaches to interrogating FlyBase. Nucleic Acids Res. 42(D1):D780-D788; http://dx.doi.org/10.1093/nar/gkt1092 [FBrf0223749]; ↑ supports the identified process or function; ↓ represses the identified process or function.

the results of experiments using genetic mutants – particularly those recently generated - can be affected by genetic background and by standard husbandry practices that provide optimal environmental conditions and select for early reproduction and short lives.[26,108,117-120] These challenges have complicated the interpretation of results examining longevity and reproduction. Discerning how these longevity mutations affect reproduction is valuable for understanding the mechanisms of age-dependent changes in reproductive performance in flies - and other metazoans - as well as the nature of physiological tradeoffs between senescence and reproduction.

The molecular chaperone 70kd heat shock protein (Hsp70) is one example of a gene involved in stress resistance with inverse effects on longevity and reproduction. Hsp70 plays critical roles in maintaining cellular homeostasis[121] and is highly conserved within the hsp family.[122,123] It confers a longevity benefit when overexpressed[124,125] and the duration of expression, and its life expanding benefits, extends well-beyond the episode of mild heat stress.[126] While increased expression of Hsp70 has an up to two-fold reduction in age-specific mortality, it also reduces embryonic viability (but not fecundity[125]). Such a trade-off is hypothesized to be the result of direct cellular interference on early-stage oogenesis from the expression of Hsp70, or indirect due to loss of resources available for oogenesis to the synthesis of Hsp70.[125] It is naturally upregulated in older (61 d pe male) flies relative to young (10 d pe male) flies[127] which may directly contribute to decreased offspring viability with increasing maternal age.

While also moderating the effects of stress, *methuselah* (*mth*), appears to have a somewhat different effect on the relationship between longevity and reproduction than *hsp70*. *mth* encodes a G protein-coupled receptor (GCPR)[128] that is involved in neuroendocrine signaling, sensorimotor function, and male germline stem cell division ([129-131], respectively). It appears to extend lifespan by mediating the effects of oxidative stress.[128] The gene product of *mth* is essential for flies as the null mutation is lethal.[128] More recently, a negative association between longevity and fertility mediated by physiological trade-offs has been observed.[118] Hypomorphic expression of *mth* is both sex- and temperature-dependent; at 29°C, lifespan increases by 18–35%[118,128] while having a negative effect on fertility.[118] At lower temperatures, hypomorphic alleles of *mth* had no effect on female lifespan and increased fertility.[118] This relationship between temperature and fertility suggests that *mth* may actually have a direct effect on reproduction which, in turn affects longevity.[118] Reduced *mth* expression *only* in the insulin producing cells (median neurosecretory cells of the brain) is sufficient to increase lifespan by 27% in females as well as increase resistance to oxidative stress without reducing fecundity.[116] Together, these results suggest that *mth*'s effects on longevity do not necessarily result in negative effects on female fecundity, but that the nature of the effect is responsive to environmental conditions. Whether or not *mth* expression changes with increasing female age is unknown.

Indy, encodes the fly homolog of a mammalian transporter of di- and tricarboxylate components of the Krebs Cycle, and is thus important in metabolism.[132] It has been suggested that decreased *Indy* expression creates a state similar to caloric restriction,[133,134] a condition that has been shown in other circumstances to extend lifespan and reduce fecundity.[135] Conditions of caloric restriction down regulate Indy mRNA, so the 2 can act in concert to extend lifespan – optimally when *Indy* expression is reduced by 25–75%.[136] Early experiments indicated that *Indy* mutants extended lifespan in several different genetic backgrounds[133] and without accompanying detrimental effects on fecundity under natural conditions suggesting that senescence and reproductive function were separable (although they were connected under conditions of caloric restriction[119]). Changes in *Indy* expression with age, perhaps as a function of age-related changes in feeding behavior, await examination. Although one examination of the gene in a controlled genetic and cytoplasmic (Wolbachia-free) environment did not detect a longevity effect in virgin flies,[120] more recent evidence continues to support its role as a longevity *and* fertility boosting gene. Naturally-occurring polymorphisms in *Indy* caused by insertion of the transposable element *Hoppel* into the first interon correspond with increased fertility and increased longevity under both normal and reduced calorie diets.[137] Interestingly, fertility is maximized when there is one copy of the *Hoppel* insertion (heterosis) and lifespan correlates positively with copy number (flies live an average of 5 d longer when heterozygotes for the *Hoppel* insertion and 8 d longer when they are homozygotes).[137] How decreased *Indy* expression promotes fertility is unknown. This tension may help maintain the polymorphism within populations. Whether or not mating and additional reproductive processes are affected by *Indy* remain unknown.

In other cases, it is already clear that genes affecting both lifespan and reproduction are members of pathways with documented roles in both processes, such as mutants involved in the insulin/insulin-like growth factor signaling (IIS) pathway. A heteroallelic mutation in the *insulin-like receptor* gene (*InR*), results in up to 85% increased lifespan, although adult flies were both dwarf and sterile.[138] Because of their roles in development, reproduction, and lifespan, hormones have been examined as possible mediators of these effects.[139] *InR* mutants produce lower levels of both ecdysone[140] and JH[138] both of which are involved in oogenesis (reviewed in[139]). Low levels of JH appear to stimulate physiological changes in the mutants resembling reproductive diapauses in wild-type flies. When administered to *InR* mutants, JH returned lifespan to normal duration and restored low-level vitellogenesis.[138] However, the lifespan extension caused by decreased JH levels occurs even in sterile females who fail to develop eggs (ovo^{D1} mutation[70]) indicating that JH's effects on lifespan are not a direct consequence of its effects on vitellogenesis and therefore do not reflect a direct trade-off between the 2 at the physiological level.

Another member of the IIS pathway, *chico*, codes for an insulin receptor substrate protein.[111] While homozygous null mutants are infertile with oogenesis arresting before vitellogenesis begins,[141,142] heterozygotes lived up to 48% longer than controls, while experiencing 17–24% reduced fecundity.[111] Reciprocal ovarian transplants between null mutant and heterozygote mutant or wild-type flies demonstrated that CHICO is necessary in the ovary for vitellogenic development.[143] The observed

lifespan extension is not solely attributable to impaired oogenesis because *chico* mutants live longer than eggless (ovo^{D1}) mutants.[111] Because INR is associated with reduced JH and ecdysteroid production, the levels of these hormones were also examined in *chico* mutants. Ecdysteroid levels in the ovary and hemolymph of mutants do not differ from wild-type flies.[143] JH biosynthesis by the corpora allata is lower in mutants at some time points (2 and 3 d pe;[144]), but not others (1 and 4 d pe;[143,144]) and JH application is not sufficient to rescue fecundity in *chico* mutants[143] continuing the uncertainty of JH's relationship with this gene. *chico's* effects on longevity appear to be via a JH- and ecdysteroid-independent component of the insulin pathway, so JH is unlikely to be a common regulator of lifespan and fertility.[143] The differing *InR* and *chico* mutant phenotypes may be explained by their activities in the PI3-kinase pathway. While both *InR* and *chico* are involved in insulin signaling through PI3-kinase, INR is able to activate PI3-kinase independent of CHICO's presence ([141,145]). In summary, while *InR* and *chico* mutants show negative relationships between longevity and fertility, the relationship is not directly attributable to changes in hormone production and evidence of a direct trade-off between the 2 remains unclear. As females age, decreasing expression of *InR*, sensitivity of INR, and/or circulating levels of CHICO might contribute to observed decreases in fecundity and fertility. However, this hypothesis is untested. Because the insulin/IGF pathway is involved in growth, reproduction, and metabolism,[146] perhaps it isn't surprising that perturbations in pathway components have varied phenotypic outcomes. The challenge will be to determine whether observed relationships reflect direct physiological connections or indirect effects mediated by common pathways.[114]

Additional protein and peptide candidates for modulators of reproductive senescence continue to emerge. One or more of the Insulin-like peptides (ILPs), ILP2, ILP3, and ILP5, are implicated in both aging and reproduction. Ablation of cells producing these 3 IPLs resulted in 18–33.5% increased female lifespan (virgin and mated females, respectively) and reducing fecundity by 25–50% between 10 and 20 d pe relative to controls.[147] Ablation also results in decreased remating rates.[148] Like *chico*, hypothetical age-related decreases in ILP could account for age-related decline in receptivity and fecundity. A conditional screen for genes promoting longevity and late-life fertility identified 2 additional promising candidates; *hebe* and *magu*.[149] Overexpression of *hebe* and *magu* resulted in 6–23% and 2–28%, respectively, increases in median lifespan and varying levels of increased fecundity later in life (30–40 d pe) and occasionally reduced fecundity earlier in life (1–20 d pe).[149] In both cases, expression in female motorneurons was sufficient for the longevity effects. These results also suggest that there is not necessarily a trade-off between longevity and reproduction. *magu* is most similar to the human SMOC2 gene, which stimulates angiogenesis[150] and is involved in GSC renewal in testes,[151] while *hebe* does not share homology with genes of known function.

Presently, there exist several viable gene candidates influencing female fertility later in life. These genes are involved in varied processes including stress resistance, metabolism, insulin signaling, as well as novel functions and highlight the complicated nature of reproductive homeostasis. Because several studies show that reproduction and longevity can be uncoupled or are prolonged in tandem, the 2 do not appear to be fixed in a direct trade-off. Future work exploring the expression patterns of these genes with increasing female age, characterizing changes in reproductive function as a result of different expression patterns, and identifying processes modulating these changes will clarify mechanisms of reproductive senescence. This, in turn, will elucidate how lifespan and reproduction are, and are not, physiologically integrated. Are the effects the result of perturbations of pleiotropic effects, shared pathways, and/or entirely separate processes?

Conclusions

Female flies continue to reproduce, although at decelerating rates, as they age. Because of this, aging females need to be considered when studying population demography. Furthermore, conserved aspects of reproductive senescence (e.g., changes in GSC proliferation, egg quality, and offspring viability) support *D. melanogaster*'s use as a model for studying reproductive senescence in other iteroparous animals. Increasing female age affects multiple components reproductive fitness including fecundity, interactions with mates, and offspring viability. While headway has been made in describing the mechanisms underlying decreased fecundity with increasing female age, dissecting the causes of other affected aspects of fertility are needed to better understand how senescence affects reproductive fitness. Current evidence suggests that reproductive performance, generally measured as fecundity, does not necessarily exist at the expense of longevity. However, the roles of genes implicated in reproductive senescence remain poorly understood. Continued efforts to describe age-related changes in gene expression, followed by mutation and misexpression studies to explore their role in reproductive senescence, will be valuable for understanding how the varied components of reproduction, behavior, fecundity and fertility, are modulated with advancing age. Documented transgenerational effects indicate that the offspring of older females may perform differently than those of younger mothers. Further exploration of the effects of offspring performance as a function of maternal (and grandmateral) age will be valuable for understanding population demography in both natural and managed populations. Finally, environmental conditions and genetic background have profound effects on gene expression and physiological responses. Therefore, understanding the effects of caloric content, diet, social environment, and temperature on reproductive senescence in multiple wild-type strains will clarify the nature of female reproductive homeostasis as well as further inform the nature of female tradeoffs among fitness components.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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