Insect oviposition site selection as a simple system to investigate the ecology and evolution of pathogen avoidance behaviour

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Summary

Pathogen avoidance is a form behavioural immunity and provides the first line of defence from infection. This article discusses insect oviposition as a tractable, mechanistically grounded model for behavioural immunity and sets a concrete research agenda linking individual egg-laying decisions to population-level disease dynamics. Because egg-laying decisions determine offspring exposure to pathogens and parasitoids, they provide a natural assay of infection avoidance with measurable impacts on fecundity, egg viability, larval survival and future infection outcomes. The sensory basis of pathogen-related egg-laying decisions is unusually well mapped in insects such as *Drosophila*, spanning olfactory detection of microbe- and parasitoid-derived cues and gustatory detection of bacterial components. We further describe how these decisions are also shaped by ecological trade-offs and social information. Egg-laying decisions are also likely heritable, opening routes to selection experiments and inference of eco-evolutionary feedbacks. Together, this article outlines how insect oviposition can link cue detection to neural and immune pathways, to individual fitness and, ultimately, to population-level transmission and pathogen evolution.

1. The importance of detecting and avoiding infection

Infection avoidance refers to host strategies that reduce the probability of contacting pathogens and parasites, acting as the first barrier in a layered defence cascade that also includes post-contact resistance and tolerance [1–3]. By lowering exposure to pathogenic sources, avoidance can substantially reduce the direct and indirect fitness costs of infection and of immune deployment itself [2–4]. Infection avoidance is expressed widely across vertebrates, invertebrates and plants from spatial, temporal and trophic avoidance, to grooming, nest hygiene and niche construction in insects, and phenology-based "disease escape" in plants [1,5]. Because avoidance reshapes contact networks, infection avoidance has ecological and evolutionary consequences for hosts and their antagonists, including reduced transmission, altered social structure, and selection on pathogen detectability and virulence[4,6].

Despite its pervasiveness, fundamental questions remain about the ecology and evolution of avoidance, such as the magnitude of among-individual variation and its genetic basis; the fitness costs of avoidance and potential trade-offs with reproduction and other defences; the sensory and neural mechanisms that transduce pathogen-derived cues into decision-making; and how environmental and social contexts modulate these processes [1,7,8]. Addressing these questions requires tractable model systems that permit causal inference from the detection of the infection cue to it the ultimate fitness consequences of avoiding infection. Insects offer such systems: they combine ecologically relevant behaviours with powerful genetic tools, well-mapped sensory repertoires for pathogen and parasitoid cues, and emerging neuro-immune pathways linking infection state to behaviour [5,7–10]. High-throughput assays and experimental control allow the quantification of fitness benefits, costs and trade-offs, while social insects permit colony-level tests of "behavioural immunity" [11–14]. In this article, we focus specifically on egg-laying behaviour in insects, and we propose that oviposition site selection can provide a tractable system in which to investigate the drivers of variation in pathogen avoidance behaviour and its ecological and evolutionary consequences.

2. Egg-laying decisions as a model of pathogen avoidance

For many insects, oviposition is the single most consequential behavioural decision shaping offspring survival, because it determines the nutritional, social and physical environment they will encounter and fixes the initial pathogen and parasite exposure of immobile eggs

and larvae [15,16] (see **Table 1**). In dipteran flies such as *Drosophila sp.*, females may lay tens of eggs per day, often on ephemeral substrates teeming with microbes, microparasites and parasitoids [15,16]. Egg-laying decisions therefore embody a natural assay of "behavioural immunity" [3,17], operating as the first line of defence by reducing contact between progeny and infectious agents [1,2]. Because oviposition is central to fecundity, yet plastically modulated by infection and risk cues, it is a powerful window into the ecology and evolution of pathogen avoidance at the interface of behaviour, sensory biology and immunity.

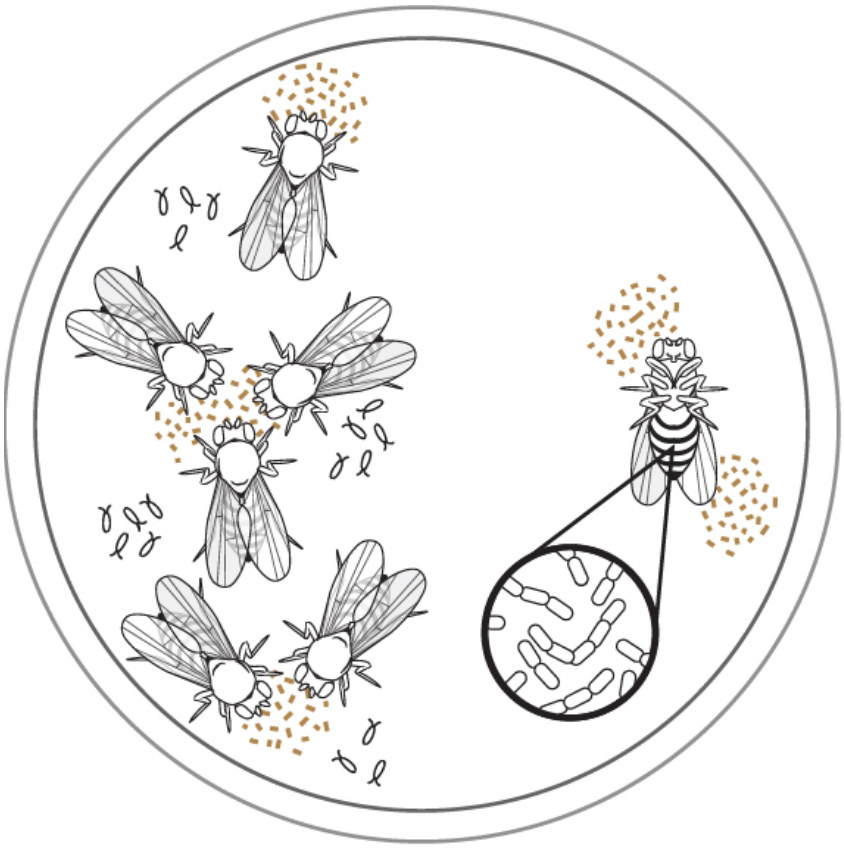


Figure 1. Where insects choose to oviposit is a major determinant of the environmental quality and infection risk faced by offspring; females should therefore be under strong selection to identify and avoid potentially infectious environments.

A key advantage of oviposition as a model for pathogen avoidance is experimental tractability (**Box 1**). Using binary or graded choice assays, it is possible to cleanly separate positional attraction/avoidance from laying decisions. Using these approaches has revealed, for example, that females frequently accept positional exposure to deterrent cues yet still avoid ovipositing there [18,19]. This sensory basis is exceptionally well mapped in *Drosophila*, enabling causal links from stimulus to receptor, circuit and behaviour [7,8,20,21].

Oviposition also lends itself to quantifying the fitness consequences of pathogen avoidance, as pathogen and parasitic exposure and infection risk can be manipulated while measuring egg number, hatch rate, larval survival and infection outcomes [20,22–24]. Oviposition choices therefore reveal the adaptive balancing of pathogen risk against other ecological pressures. Females integrate abiotic, biotic, and social information (**Table 1**) and frequently resolve trade-offs by either accepting some risk to gain nutritional or competitive advantages for offspring or switching to alternative defence strategies (e.g. medicative oviposition on ethanol to reduce parasitoid success [22]).

3. Pathogenic cues influencing oviposition site selection

Female insects weigh an array of abiotic and biotic cues, many of which are directly informative about pathogen risk (Table 1) and will frequently avoid oviposition on substrates emitting microbial “danger” volatiles. A common example is geosmin, a microbial toxin cue detected by *Or56a* olfactory neurons that elicits robust aversion, overriding attraction to food odours [20]. Phenol, abundant in carnivore faeces and produced by pathogenic bacteria, similarly induces egg-laying aversion through *Or46a* on the maxillary palps [25]. Fungal 1-octen-3-ol also repels oviposition and is being explored for pest management, showing reduced egg loads per fruit in *D. suzukii* field assays [26] (see also **Table 1**).

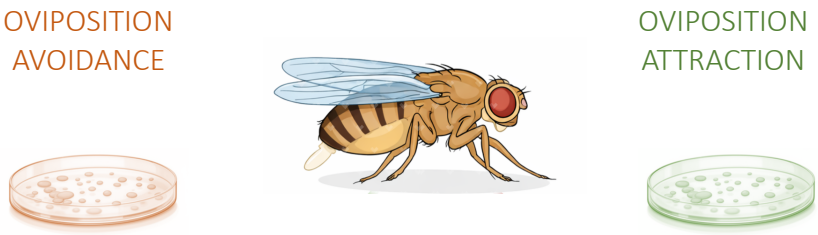
Beyond pathogen-derived volatiles, bacterial cell-wall components provide potent contact cues: bacterial lipopolysaccharide (LPS) in food triggers oviposition and feeding avoidance via *TRPA1*-mediated gustation, while LPS on appendages induces hygienic grooming [27–29]. Parasitoid risk is detected both directly and indirectly. *D. melanogaster* avoids ovipositing in patches laced with the *Leptopilina* sex pheromone iridomyrmecin via *Or49a*, with co-expressed *Or85f* decoding additional wasp volatiles [30].

When avoidance is constrained, females may switch to “medicative” oviposition, by laying eggs preferentially on ethanol-rich substrates that protect larvae from parasitoids [22], or to citrus substrates, whose terpenes repel wasps [23]. Ectoparasites elicit similar trade-offs between avoidance and medicative responses: *D. nigrospiracula* avoids mite-contaminated patches, yet when food is limiting will still lay more eggs on mite-exposed cactus than on poorer alternatives[31](**Table 1**).

It is important to note that not all microbial cues signal danger. Females also exploit microbial metabolism to locate nutritionally favourable sites for larvae. *In D. melanogaster*, sucrose depletion by *Enterococcus* on fruit creates an oviposition cue; females prefer these patches even though they do not prefer the bacteria per se, indicating that they assess microbial

modification of the substrate rather than microbial presence alone [32], consistent with adaptive foraging for offspring [16].

Table 1. Summary of literature on oviposition site selection in *Drosophila*



		Substrate		Sensory system
ABIOTIC	Flies avoided high levels of AA, showing a trade-off between oviposition and positional preference.	Acetic acid (AA)[18]	Strong oviposition preference for sites with acetic acid but a positional avoidance to it.	Gustatory (taste neurons on the labellum)
		Breeding substrate *Vitis vinifera* (grape) and *Cydonia oblonga* (quince)[33]	Laid a greater proportion of their eggs on the grape. However, flies reared on quince had greater larval viability and developed faster.	N/A
		Lobeline (bitter compound)[19]	When laying eggs females prefer surfaces with lobeline.	Gustatory Gr66a receptor on the internal mouthparts lining the pharynx.
		Acid-containing sugar[34]	Females preferred to lay eggs on acid-containing sugar-agar rather than just sugar alone.	Gustatory (sour gustatory receptor neurons expressing IR76b and IR25a)
	Blind females were capable of avoiding UV light when laying eggs.	UV light[35]		Vision and gustatory (bitter-sensing neurons expressing H2O2-sensitive dTRPA1 isoforms)
MICROBE	Avoidance of Bacterial-derived phenol within carnivore faeces.	Bacteria-associated phenol[25]		Olfactory (Or46a receptor on neurons)

	Avoidance of LPS and *E.coli*.	*Escherichia coli* and associated lipopolysaccharide [27]		Gustatory neurons expressing Gr66a bitter receptors causing TRPA1 activation.
	Preference for sites without these compounds and in the field octenol treatment reduced the number of eggs per fruit.	Geosmin and 1-octen-3-ol produced by many harmful fungi, bacteria, and cyanobacteria [26]		N/A
	Avoidance of pathogenic mold *P.expansum*.	Beneficial bacteria (Enterococci) [32]	Preference of commensal species (*L.plantarus, Lactococcus, Weisssela, Saccharomyces, Acetobacter*) but Preference was positively correlated with sucrose consumption by microbes within decomposing fruit.	Sweet gustatory receptors Gr5a and Gr64a.
	Females preferred egg-laying near clean fly carcass, avoiding DCV-infected carcass.	Carcass infected with Virus (Drosophila C Virus)[24]	DCV-infected females did not have a preference between infectious and non-infectious carcasses, though avoided sites with no carcass.	N/A
	D.suzukii avoided oviposition on sites with microbes.	Commensal microbes transferred by other flies (most commonly *Acetobacter* and *Gluconobacter) [36]*	*D. melanogaster* and D.*diarmipes* preferred ovipositing on sites with microbes.	N/A
	Avoided and laid fewer eggs on *Bacillus thuringiensis* bioinsecticide.	Bacteria (*Bacillus thuringiensis*) and associated products (bacterial spores and toxins) [37]		N/A
ECTOPARASITE	Avoidance of sites with wasps, however when forced to lay eggs when wasps were present D.*melanogaster* reduced the number of eggs laid.	Parasitic wasps (*Leptopilina boulardi*)[38]		N/A

		Parasitic wasps (*Leptopilina heterotoma*) and alcohol[22]	Females switch to laying eggs where there are toxic levels of alcohol which can protect offspring from infection when wasps are present.	Visual
	Larvae avoided sites, and females avoided laying eggs on sites with parasitoid wasp odours.	Odours (iridomyrmecin, actinidine and nepetalactol) produced by *Leptopilina* wasps [30]		Olfactory Or49a and Or85f receptors
	Flies avoided surfaces containing mites.	Parasitic mites (*Macrocheles subbadius*) vs food source and presence of other females' eggs [31]	Flies laid more eggs on sites with cactus (food source) and mites than sites with no mites or cactus. Females prefer sites with other eggs present despite these sites being exposed to mites.	N/A
CONSPECIFICS	.	Mated vs virgin females [39]	Females laid more eggs in the presence of previously mated females and their eggs than when in the presence of virgin females and unfertilised eggs	N/A
		Pheromone cues from mated females [40]	Females were attracted to cues released by mated females that mark high quality patches.	N/A
	Avoided egg-laying if pheromone concentrations too high (signalling competition) and too low (not signalling benefits of group laying).	Pheromones deposited by males and mated females (11-cis-vaccenyl acetate (cVA) and 7-Tricosene.[41]	Preference for oviposition at Intermediate pheromone concentrations.	Olfactory receptors Ord67d and Or65a
		Conspecific and heterospecific larvae and eggs.[42]	Preference for ovipositing on surfaces with eggs and larvae than without.	N/A

4. Life-history trade-offs arising from pathogen-induced egg-laying decisions

The examples above provide compelling evidence that pathogen avoidance in oviposition delivers fitness benefits by lowering offspring exposure and mortality, and by steering laying towards substrates that mitigate risk. Although the strongest quantitative demonstrations of fitness gain currently come from parasitoid systems [22,23,30,43], the convergence of dedicated danger-detection pathways with consistent behavioural outputs supports the view that pathogen-related oviposition choices are adaptive. Yet, pathogen avoidance during oviposition is unlikely to be cost-free, and several lines of evidence point to ecological contingencies and trade-offs that can constrain the evolution of extreme avoidance.

Behavioural architecture can itself impose opportunity costs. In *D. melanogaster*, positional attraction or aversion can be decoupled from the decision to lay, such that females may enter and sample risky patches before withholding oviposition [18,19]. This strategy increases search time and movement, potentially elevating energetic expenditure and exposure to other hazards. Likewise, danger cues can induce hygienic grooming and active sampling [29], behaviours that are protective but also time- and energy-consuming [16]. A further potential cost may arise through social information (**see section 6**), which while often beneficial, can also generate costs through density-dependence [44]. For example, pheromonal blends deposited by males and mated females bias oviposition in a concentration-dependent fashion, with intermediate levels preferred and high levels avoided. This is consistent with females steering away from congested sites where competition and pathogen transmission might be higher [21,40,41](see also Table 1).

Another common trade-off faced during egg-laying decisions occurs between safety and resource quality. When confronted with a choice between safer but nutritionally suboptimal sites and risky but high-quality food, females may prioritise offspring nutrition as described above for *Drosophila nigrospiracula*, laying more eggs on mite-exposed cactus when alternative substrates are poor [31]. Similar outcomes are observed in *D. melanogaster* where females will preferentially lay eggs surrounding a fly carcass relative to a simple food substrate, even when the carcass is contaminated with Drosophila C Virus; in this case, larvae cannibalising the carcass experience benefits in egg viability (presumably because the carcass offers additional nutrition) and these benefits outweigh the potential costs of viral infection [24]. Species differences in the weighting of mechanical versus microbial cues also echo such trade-offs. The invasive *D. suzukii* de-emphasises microbial films typical of rotting fruit and instead prefers hard, intact substrates that resemble ripe fruit, a strategy

that likely reduces exposure to saprophytic microbes but may forgo commensal services that can aid larval development [36]. Even within a cue class, preferences tend to peak at intermediate values, as in the case of acetic acid: *D. melanogaster* shows strong oviposition preference for acid-containing substrates but aversion at high concentrations, implying diminishing returns or toxicity costs at extremes [18,34].

Trade-offs also extend to physiology. Systemic infection down-regulates oviposition via peptidoglycan sensing and NF-*κ*B activation in octopaminergic brain neurons, a neuroimmune checkpoint that reallocates resources from reproduction to defence [8,45]. While beneficial in the short term at the level of an infected individual, such immune-reproductive crosstalk imposes a fecundity cost. More generally, ecological constraints can favour intermediate strategies when predators or pathogens impose opposing risks, or when safe sites are rare in heterogeneous landscapes [4]. Evidence for explicit genetic trade-offs between oviposition avoidance and immune competence remains sparse [46], but some theory and comparative work on avoidance predict that energetic, opportunity, and reproductive costs can maintain variation in avoidance within populations [2,47,48].

A priority for the field is to scale laboratory assays to semi-natural settings to link decisions to infection outcomes and lifetime fitness under realistic ecological complexity. Oviposition systems are well suited to quantifying these trade-offs by employing controlled assays to jointly measure laying rate, search time, egg-to-adult survival and development, under factorial manipulation of risk and resource quality (Box 1). Mechanistically, immune–reproductive trade-offs can be probed by activating innate pathways and assaying consequent oviposition suppression via octopaminergic circuits, thereby linking costs mechanistically. Further, comparing reaction norms across genotype will reveal whether the costs experienced by avoiding infection may be sufficient to maintain standing variation in avoidance.

5. Evolutionary and quantitative genetics of pathogen-related oviposition choices

While the sections above focus on the causes of variation in pathogen-related egg laying decisions, it is also important to investigate the ecological and evolutionary consequences of avoiding infection. Given the trade-offs between resource acquisition and avoiding detrimental infection, a key question is how such trade-offs may impact the evolution of such avoidance strategies. This question presupposes that egg-laying decisions are amenable to respond to selection, which while likely, requires the demonstration of two basic conditions [49]: heritable genetic variation in pathogen-related egg-laying decisions, and that this

variation is associated with clear fitness costs and benefits. It is therefore essential to measure the level of phenotypic variation in egg-laying choices present in natural insect populations, and to quantify how much of this variation is broadly explained by genetics (the broad-sense heritability, H^2).

Recent work has found heritable genetic variation for avoidance behaviours, including trophic avoidance in *D. melanogaster* [50] and in the nematode *C. elegans* [51]. More generally genetic variability for most forms of pathogen avoidance remains surprisingly undermeasured, and the proportion attributable specifically to oviposition decisions in response to pathogen cues remains unknown. In *D. melanogaster*, this can be addressed with iso-female lines or reference panels (e.g. DGRP [52]), using replicated two-choice assays either with live pathogens or with pathogen-associated cues (e.g. geosmin or LPS), to estimate repeatability and broad-/narrow-sense heritability. Cross-environment designs using benign vs risky substrates will further reveal genetic variance in plasticity (G×E), a key ingredient for adaptation to heterogeneous risk landscapes [2,4].

If we assume that oviposition-related infection avoidance is even weakly heritable, the question that follows is how it might be shaped by natural selection. Whether avoidance is under directional, stabilising or disruptive selection will depend on spatio-temporal heterogeneity in risk and resource quality [4]. Again, insects offer powerful and tractable systems. By imposing gradients of pathogen or parasitoid risk across oviposition patches in mesocosms, it is possible to track maternal choices and offspring survival and estimate selection gradients on cue sensitivity [53]. Stabilising selection is predicted when extreme avoidance incurs opportunity costs (e.g. poor nutrition or prolonged search), whereas disruptive selection may emerge when alternative strategies (risk-tolerant vs risk-averse) perform best in different microhabitats (**Figure 2**).

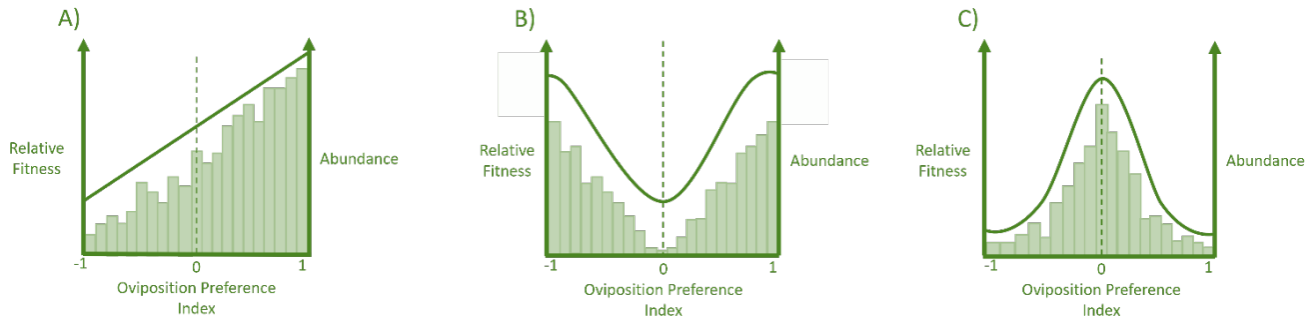


Figure 2. Phenotypic selection of oviposition preference index. Graphs show the hypothetical standardised fitness (black line) and the abundance (grey bars) of oviposition preference index in a hypothetical population. There are three possible forms of natural selection: (A) directional, (B) disruptive, and (C) stabilising. When an extreme trait value has the highest fitness, for example a high oviposition preference index where an individual that exclusively avoids the pathogen has the highest fitness (e.g. most surviving offspring) the form of selection acting on this distribution is characterised as directional selection (A). Disruptive selection is characterised by a distribution of two extreme values having the highest fitness with an intermediate oviposition index selected against due to possessing a lower fitness (B). Stabilising selection is categorised by a middle, intermediate value that is associated with the highest fitness (C). Dotted line depicts an intermediate preference of 0, -1 describes a complete preference for pathogen whilst 1 describes a complete avoidance of pathogen.

What are the direct and correlated responses to selection for extreme pathogen-related oviposition choices? Artificial selection for extreme avoidance (or attraction) to defined pathogen cues provides a causal route to evolutionary inference. For instance, selecting insect lines for high vs low oviposition aversion to geosmin, parasitoid odours, or LPS should yield rapid direct responses, while correlated responses can be measured in fecundity, larval survival, and immune phenotypes (e.g. antimicrobial peptide expression, haemocyte activity). Such experiments would enable testing for shared genetic architecture with other defences and can reveal sensory or neuro-immune pleiotropy. In *Drosophila*, further whole-genome and transcriptomic comparisons between selected lines could identify candidate receptors and circuits underpinning evolved changes.

In addition to understanding the evolutionary drivers of variation in pathogen-related oviposition choices, avoiding infection will likely cause important co-evolutionary feedbacks. Avoidance can depress transmission by reducing contact rates and alter social and spatial structure, thereby feeding back on pathogen and parasitoid evolution [4]. If hosts avoid substrates that advertise danger, selection may favour pathogens that reduce detectable cues or altered virulence if detectability covaries with pathology [6,54]. These feedbacks are testable in insects. By evolving bacteria or fungi under regimes where host oviposition

avoids microbially scented patches it is possible to then test whether pathogen cue production declines.

6. Social modulation of pathogen-related oviposition choices

Oviposition choices are shaped by social cues that can either amplify or override pathogen-related information. Females are known to copy conspecifics' choices, laying more where experienced females have laid than when encountering the same substrates alone [39], and they prefer patches marked by mated females, which act as pheromonal indicators of high-quality sites [40]. Males contribute marks too: food odours trigger deposition of 9-tricosene, which guides aggregation and female oviposition via OR7a [21]. Responses to blends of cues are concentration dependent, as combinations of cis-vaccenyl acetate, 7-tricosene and its oxidation product heptanal, sensed by OR67d/OR65a pathways, can either attract or deter laying depending on signal strength and perceived competition [41] (Table 1).

Social transmission can propagate or even magnify pathogen- or parasite-induced shifts in oviposition. A well-studied case is parasitoid wasp exposure: *D. melanogaster* females that see wasps switch to ovipositing on ethanol-rich substrates, which "medicates" larvae against parasitism; critically, exposed females also socially transmit this oviposition strategy to naive conspecifics via visual cues and neuropeptide F-dependent circuits [22,55]. More generally, insects often use social information to avoid disease risk: individuals reduce association with sick conspecifics and adjust space use in response to infection cues [56,57], and social learning can bias food and oviposition preferences toward substrates associated with successful conspecific reproduction and beneficial microbial communities [39,58]. Together, these studies show that group-derived information can both disseminate pathogen-avoidance strategies and, in some contexts, outweigh direct risk cues when social indicators of site quality are strong.

7. Future directions

We have highlighted how oviposition systems can link cue detection to neural and immune pathways, to individual fitness and, ultimately, to population-level transmission and pathogen evolution. In the process, we have identified five major questions where insects offer promising experimental tractability:

(i) quantify standing genetic variation, heritability and G×E for pathogen-related oviposition in natural populations;

(ii) test for correlated responses to selection with immune traits;

(iii) measure the full fitness landscape of avoidance by jointly manipulating risk and resource quality in semi-natural mesocosms and estimating selection gradients;
(iv) dissect how social information modulates avoidance across densities and group compositions;
(v) test co-evolutionary feedbacks by evolving pathogens under host avoidance regimes to assess changes in cue production and virulence.

Addressing these questions is not only important to our fundamental knowledge of infection ecology and evolution but also offers substantial applied value. Pest management, for example, can exploit dedicated “danger” circuits by using approaches that combine pathogen-associated repellents (such as geosmin, phenol, 1-octen-3-ol) to deter pest egg-laying, in addition to microbial or conspecific-derived attractants to concentrate oviposition in lethal sinks. Non-linear and context-dependent responses. Harnessing medicative preferences (e.g., ethanol) may further divert oviposition to treated substrates. If, as we hypothesise, avoidance can select for reduced cue detectability, rotating cues and integrating behavioural surveillance will be essential to slow behavioural resistance. By uniting mechanism with fitness and ecology, insect oviposition systems can deliver a predictive, evolution-aware toolkit for managing disease risk and agricultural pests.

Box 1. Methodological guidance for oviposition choice assays

Designing robust assays

Two-choice oviposition assays are powerful but sensitive to design details. Female state is a major source of variance, so it important to standardise age, mating status, nutritional state and egg load. Like most behavioural assays, CO2 anaesthesia immediately prior to testing should be avoided because it is likely to alter behaviour [59]. Oviposition and activity are circadian-gated, so the light:dark cycle and time-of-day should be controlled and reported [60,61]. Because idiosyncratic side biases are common, the side carrying the focal cue should be randomized across arenas and across days [20,27]. A recurring issue is that where females lay can be influenced as much by where they spend time as by any active evaluation of substrates. It therefore helps to quantify both positional preference and laying decisions by recording time on each substrate alongside egg counts [18,19].

Stimulus delivery and contamination

The way pathogenic stimuli is delivered is important. Behavioural tests are more interpretable when cue concentrations fall within physiologically relevant dynamic ranges. For contact cues (e.g., LPS), it's important to incorporate the cue well into the laying substrate, as is confirming the viability or heat-killed status of microbial preparations. Carry-over of pheromones is a frequent, often hidden contaminant: thorough cleaning between trials and replacement of substrates reduces biases caused by male-deposited hydrocarbons, which affect aggregation and oviposition choices [21,40].

Single versus group assays

A common experimental design decision is whether to test oviposition using single insects or groups of females. Single-female assays provide independent replicates, allow estimation of among-individual variance and repeatability, and minimise social feedback which can often confound accurate estimates of "preference". Group assays, conversely, boost throughput and can deliberately capture social information use, but they introduce non-independence through social copying, pheromonal marking, and density effects; in these cases the arena, not the individual, should be treated as the unit of replication [13,21,39]. In some cases, video-tracking can be employed to record interaction structure and individual trajectories in group contexts, which can enable explicit modelling of social effects [62–64].

Repeatability, power and analysis

Reporting assay repeatability within and across days improves the robustness of effects; re-running a subset of females allows estimation of within-individual consistency [65]. Data analyses should leverage the native scale of the data: when egg counts are available, it's preferable to avoid ratios in favour of binomial or related models of counts (eggs on cue vs total) using GLMMs with random effects for arena, batch/day, and, where applicable, individual identity [66,67]. Overdispersion and zero-inflation are common in egg-count data, especially when many females lay few or no eggs, so diagnostics are important, and an observation-level random effect can be added where needed [68]. Classic preference summaries such as *Oviposition Index = (A−B)/(A+B)* are useful for visualization and comparison [18], but formal analyses are best based on the underlying counts. Defining minimal egg thresholds per trial a priori and ensuring sufficient replication at each treatment level help to analyse interaction terms with adequate power.

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