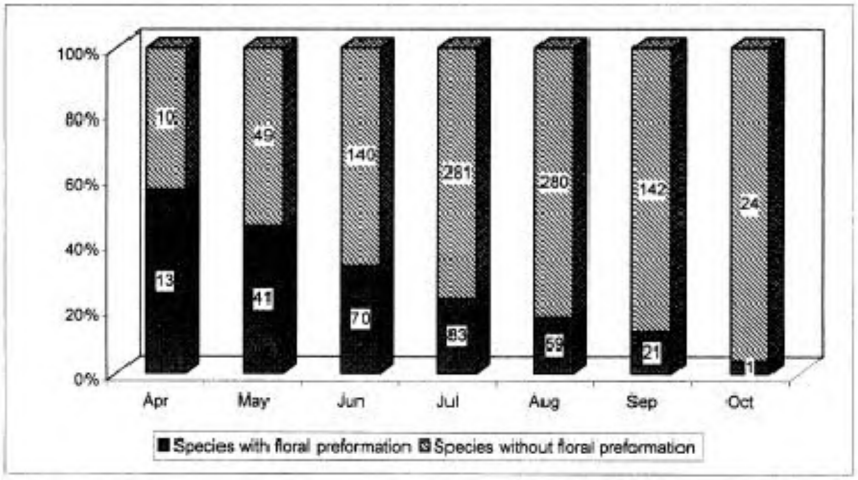

Figure 2. Per cent share of species with floral preformation in overall flowering.

initiate growth even below a few centimetres of snow cover, a phenomenon also found in species of *Ranunculs*, *Caltha*, *Soldanella*, *Erythronium* elsewhere, which is believed to be possible because plants are able to perceive minute amounts of radiation and learn from these the time of the day, time of the year and whether it is appropriate (from evolutionary experience) to initiate a certain developmental phase of their life[2].

The level of differentiation is high in some species like *Caltha palustris*, *O. polypetala*, *T. acaulis*, *Primula* spp., *Rhododendron* spp., *Corydalis* spp., *Cassiope fastigiata*, *Bergenia stracheyi*, *Podophyllum hexandrum*, *Gaultheria trichophylla*, *Malaxis* spp., *Dactylorhiza hatagirea*, *Goodyera fusca*, *Aletris pauciflora*, etc. and flowers with floral parts, inflorescence or whole aerial parts are preformed as miniatures inside winter buds. Sometimes, as in *B. stracheyi*, even the true colour of the petals is visible. Another interesting case is that of *Malaxis* spp. which have a basal pseudobulb, one or few basal leaves and a solitary, erect spike of few flowers. This whole structure of the plant body is developed in miniature form in a side groove of the pseudobulb of the parent plant and remains well protected by basal sheaths.

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A potential mass-scale assay for level of immune competence in *Drosophila*

It is now increasingly being recognized that investment in immune functions is likely to trade-off with major life-history-related traits such as lifespan, fecundity, competitive ability, or resistance to abiotic stress, and that knowledge of immune system competence is, therefore, important to our understanding of life-history evolution[1–5]. At this time, *Drosophila* is probably the best-understood model system in terms of physiology, genetic architecture and evolution of life-history-related traits[6]. Recently, the immune system of *D. melanogaster* has also been extensively studied, and many molecular details of pathways involved in the production of specific antimicrobial peptides as a response to infection by various types of microbial pathogens are now known[7–9]. Immune responses of *Drosophila* larvae to attack by parasitoid wasps, a common natural enemy, have also been studied in some detail, including attempts to understand the costs, in terms of fitness-related traits, to mounting immune defences against parasitoid attack[3,4]. Given that many of the commonly studied *Drosophila* species breed in decaying organic matter, microbial pathogens are likely to be an important selective agent, especially in tropical

areas. Yet, there are few studies with *Drosophila* that try to link immune responses to microbial pathogens with other life-history-related traits and, in general, incorporate costs of immune system competence into studies of life-history tradeoffs[2].

One potential reason for the paucity of studies linking immune function and life-history-related traits in *Drosophila* is that the assays of the different components of immune response to microbes, such as rate of clearing of the pathogen[2], extent of phagocytosis[10], or levels of antimicrobial peptides induced[11], are not conveniently done on a mass scale on large number of flies, whereas for rigorous life-history studies, some kind of mass-scale assay of level of immune function would be desirable, such that large numbers of populations could be screened for immune function levels in various environments. Similarly, the standard technique of infecting flies by pricking them with a micro-needle dipped in the pathogen culture[2,8,10,11] is too tedious to lend itself to mass-scale assays of immune function levels in response to infection. Moreover, in the context of life-history evolution, what one would like to know is not so much the details of the proximal immune response to infection, but rather its ultimate consequences in terms of its effect on traits related to fitness. Consequently, we decided to examine whether merely rearing *Drosophila* adults in the presence of *Escherichia coli* – a pathogen known to elicit an immune response[10,11] and to affect mortality[2] in *Drosophila* following injection – would produce a detectable increase in mortality. If so, we wanted to further ascertain whether *E. coli* induced a greater increase in mortality in long-term laboratory populations, relative to recently wild caught populations, as laboratory populations are expected to have been under relaxed selection for maintaining high immune competence for many generations in benign laboratory conditions. Thus, if wild caught populations do indeed show relatively smaller increases in mortality due to *E. coli* than long-term laboratory populations, assays of survivorship following exposure to *E. coli* (or other pathogens) could be used as a surrogate for levels of immune competence, the advantage being that large numbers of flies could be screened in such assays, compared to the typically used methods.

We assayed time-to-death in the presence and absence of growing *E. coli* cultures in three populations of *Drosophila* spp. One was a population of *D. melanogaster* that had been reared in the laboratory for over 700 generations (population JB-1, first described by Sheeba *et al.*[12]). The other two were populations of *D. ananassae*, which also belongs to the melanogaster species group. One population, AB-1, had been reared in the laboratory for 40 generations, whereas the other, AW, was a more recently caught wild population that had been in the laboratory for only eight generations at the time of the study. Eggs from each population were distributed into vials (9 cm × 2.4 cm) at a density of 60–80 eggs per vial, and freshly eclosed (within 6 h post-eclosion) adults from these vials were used to set up the assay. For the assay, 60 vials were set up with 3 ml of Luria Bertani (LB) agar medium on which *E. coli* strain DH5α was streaked. These vials were then incubated at 37°C for 24 h to allow bacterial growth. At this point a lawn of bacteria was visible on the surface of the medium in the vials. Sixty control vials containing LB agar were also set up in a similar manner, except that they were not inoculated with *E. coli*. To set up the assay, either five freshly eclosed males or five freshly eclosed females were placed into a vial. Ten control vials and ten vials with *E. coli* were set up for each population × sex combination, and kept at ~25°C under constant light. The vials were observed every 2 h, and the death of any fly during the previous 2 h period was recorded. This process was continued until all 600 flies had died.

Overall, JB-1 flies had a far lower mean time-to-death compared to either AB-1 or AW flies (Figure 1); the reason is not clear at this time. For analysing the effect of *E. coli* on time-to-death across populations and sexes, we transformed the primary data on time-to-death of individuals in the vials with *E. coli* by dividing it by the mean time-to-death averaged across the control vials for that particular population × sex combination. Thus, if X_{2ijkl} were the time-to-death of the lth individual in the kth vial of the jth sex from the ith population from the 2nd treatment (with *E. coli*), we considered for analysis the transformed variable $Y_{2ijkl} = X_{2ijkl}/X_{1ij\bullet\bullet}$, where $X_{1ij\bullet\bullet}$ was the mean time-to-death, averaged over individuals and vials, for the combination of jth sex and ith population from the 1st treatment (control). Y_{2ijkl}, therefore, reflects the effect of *E. coli* in enhancing mortality rate, as it is the time-to-death in the presence of *E. coli* as a fraction of the average time-to-death for that sex and population in the control vials. Higher values of Y_{2ijkl}, thus, would reflect relatively greater degree of resistance to the pathogenic effect of *E. coli*.

Analysis of variance on the transformed variable Y_{2ijkl} revealed significant main effects of population ($F_{2,54} = 12.99$, $P < 0.0001$) and sex ($F_{1,54} = 6.66$, $P = 0.01$), but no significant population × sex interaction ($F_{2,54} = 0.78$, $P = 0.46$). On

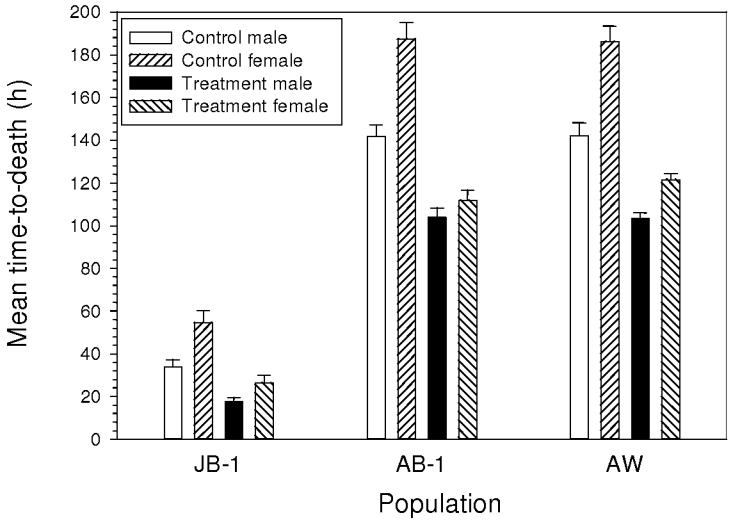

Figure 1. Mean (± SE) time-to-death in males and females of the three populations in treatment (*E. coli*) and control (no *E. coli*) vials.

an average, females and males lived only 58 and 66% as long, respectively, in the presence of *E. coli* as they did in control vials. Multiple comparisons by Tukey's Honest Significant Difference test indicated that the effect of *E. coli* on time-to-death did not vary significantly between the two *D. ananassae* populations (AB-1 and AW), whereas the effect of *E. coli* on time-to-death in the *D. melanogaster* population (JB-1) was significantly ($P < 0.001$) greater than that on either AB-1 or AW. Flies from populations AB-1 and AW lived 67 and 69% as long respectively, in the presence of *E. coli* as they did in control vials, whereas those from JB-1 lived only 50% as long in the presence of *E. coli* as they did in control vials.

It is clear from the results that adult *Drosophila*, in the presence of a growing *E. coli* culture, suffered increased mortality, leading to a reduced mean time-to-death. The detrimental effect of *E. coli* was significantly more severe on the long-term laboratory population of *D. melanogaster* (JB-1), than on the two relatively recently wild caught populations of *D. ananassae*. This is likely to be due to the fact that the JB-1 flies, over the course of several hundred generations of rearing in optimal laboratory conditions, have become less competent at mounting an immune response to *E. coli* than wild caught species which, presumably, need to deal with exposure to microbial pathogens in their natural habitat, which largely consists of rotting fruits, vegetables and domestic kitchen garbage. The fact that the two *D. ananassae* (AB-1 and AW) populations did not differ significantly in their susceptibility to *E. coli* suggests that the additional 32 generations of laboratory rearing undergone by AB-1 relative to AW have not yet resulted in a discernible loss of immune function.

The above inferences are, of course, predicated on the increased mortality due to *E. coli* cultures being the result of pathogenic effect following infection. Given that *E. coli* is known to elicit immune responses[10,11], increase mortality[2] and multiply within the flies[2] after injection; the assumption of a pathogenic effect is not unreasonable, provided the bacteria actually entered the bodies of the flies during the time-to-death assay. Therefore, we tried to examine whether simply keeping flies in vials with *E. coli* growing on the LB agar actually led to the bacteria entering the body of the flies. We first ascertained that washing flies surface-contaminated with *E. coli* alternately in 70% ethanol and distilled water suffices to get rid of all surface contamination. Anaesthetized flies were rolled around with a brush for 30 s on a lawn of growing *E. coli* on a petri plate. The flies were then subjected to three rounds of alternate washing in 70% ethanol (30 min) and distilled water (30 min). After the third wash, individual flies were crushed in Insect Ringer's Solution, mixed, and the supernatant plated on LB agar plates and incubated for 24 h at 37°C. The plates were then examined for bacterial growth, and compared with plates streaked with supernatant from flies rolled on *E. coli* but not subjected to washing. Five such plates each, streaked with supernatant from washed versus unwashed flies, were observed, and in all cases washed flies did not result in growth on the LB agar plates after streaking, whereas plates streaked with supernatant from unwashed flies showed good amount of *E. coli* growth (Figure 2). We then set up vials of LB agar with or without *E. coli* growing in them, as in the time-to-death assay. Flies were introduced into these vials and left in them for 24 h. Next, the flies were subjected to three rounds of alternate washing in 70% ethanol (30 min) and distilled water (30 min), crushed in Insect Ringer's Solution and plated, as described above. Extensive *E. coli* growth was seen in all five petri plates streaked with flies coming from LB agar vials with *E. coli*, whereas none of the five petri plates streaked with flies coming from LB agar vials without *E. coli* showed any *E. coli* growth (Figure 2). This observation confirmed that merely keeping flies in vials with *E. coli* growing on the LB agar surface results in *E. coli* entering the body of the flies, possibly by ingestion or through the tracheal openings, or both.

Examination of dead flies did not suggest that getting stuck in the vials with

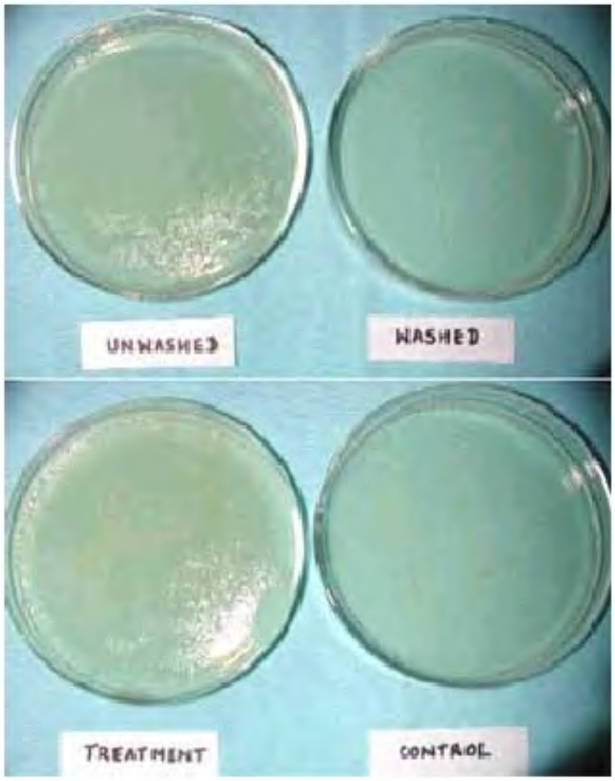

Figure 2. A pair of representative petri plates from the two experiments to ascertain whether simply keeping flies in vials with *E. coli* growing on LB agar actually led to the bacteria entering the body of the flies. (Top panel) 'Unwashed' and 'washed' refer to plates streaked with supernatant from crushed flies that had been originally rolled in *E. coli* to surface-contaminate them, and were subsequently subjected to no washing or three rounds of washing in ethanol and distilled water respectively. *E. coli* growth can be seen in the 'unwashed' but not the 'washed' plate. (Bottom panel) 'Treatment' and 'control' refer to flies kept in vials with or without *E. coli* growing on the LB agar respectively. Both 'treatment' and 'control' flies were washed in ethanol and water, crushed in Insect Ringer's Solution, and then streaked onto plates. *E. coli* growth can be seen in the 'treatment' but not the 'control' plate.

growing bacterial culture was a cause of mortality, further supporting the view that the mortality effects of rearing in the presence of *E. coli* are due to infection. Moreover, these species of *Drosophila* are microbe (yeast) feeders and it is, therefore, likely that they would ingest bacteria in the course of feeding. We hope to settle the issue unequivocally in the future by showing that rearing *Drosophila* in the presence of *E. coli* not only results in infection, but also elicits an immune response by correlating the degree of mortality induced with the magnitude of immune response mounted. At this point, we believe that our results, though somewhat preliminary in nature, suggest that growing *Drosophila* in the presence of microbial pathogens could provide the basis for convenient mass-scale assays of both, the level of immune competence and the effects of infection on various life-history-related traits. The development of such mass-scale assays will be helpful in empirically studying the immune function in the context of life-history evolution in *Drosophila*.

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Late Holocene palaeo-winds and climatic changes in Eastern Antarctica as indicated by long-distance transported pollen–spores and local microbiota in polar lake core sediments

The continent's hostile climate precludes most terrestrial life out of which mosses and lichens are the most widespread vegetation where the ground is exposed and moisture is available. In the Schirmacher oasis the precambrian crystalline basement of East Antarctic platform is exposed over an area of about 30 sq km. The polymetamorphic rock sequence consisting dominantly of biotite-garnet gneiss and pyroxene-bearing granulites with minor intercalation of marbles and calc-silicate rocks, ultramafics, amphibolites and other metabasites is traversed by a number of distinctly younger basalt (dolerite) dykes[1]. The rocks have undergone multiple episodes of metamorphisms, migmatization and deformation.

The lakes, ponds and pools in the lake district of Schirmacher oasis cover a large area representing the essential part of the surface water. The elevation of the oasis ranges between 0 and 228 m with an average of about 100 m. The gentle slopes and plain areas are covered with mostly a thin blanket of moraine matrix.

Only existing plant lives, namely Mosses – *Polytrichum alpinum*, *Drepanocladus uncinatus*, aquatic algae and luxuriant growth of lichens both crustose type (*Acarospora*, *Rhizocarpon*, etc.) and foliose type (*Umbilicaria*) are found on exposed pattern ground, rocky stratum and lakes. *Deschampsia antarctica* (Fam. Poaceae) and *Colobanthus quitensis* (family Caryophyllaceae) are the only higher groups of plants found in Antarctic peninsula. The aim of this paper is to understand the distribution of palynodebris in Antarctic surface deposits in the first phase and subsequently the transmitted pollen-spore data could be used in deciphering Holocene climatic oscillations in lake sediment of Polar region. The present data support the earlier studies on transport of exotic palynomorphs into the Antarctic region[2–7].

Palynological samples including moss tufts and lichen patches, were procured from near lake sites, dry valleys and nunatak (Veteheia) around Schirmacher oasis of Eastern Antarctica (Figure 1). Fifty grams of moss soils and 5–10 g of lichen soil were taken for pollen analysis to assess accurate relative frequencies of various microbiota in the measured sediment. Two sediment profiles (50–60 cm) were collected, one each from Zub lake (70°45′39.4″S : 11°44′8.6″E) and Long lake (70°45′20″S : 11°4′E) during the XX Indian Scientific Expedition for palynostratigraphical studies (Figure 2). Sediment samples were collected in the interval of 5 cm in each case using a HYDROBIOS gravity corer (Kiel, Germany) for palynological studies, and for