Proposed title: *Using flight initiation experiments to investigate whether predation risk is a contributing factor to seasonal polyphenism in Hetaerina titia wing colouration.*

Key terminology: Interspecific interaction, intraspecific interaction, heterospecific, conspecific, phenotypic plasticity, polyphenism.

Background*:* The complex intricacy of interactions within ecosystems means that there will unfailingly be competition for shared resources. The proximate cause of interspecific and intraspecific aggression is usually competition to access these finite, shared resources[1]. Specifically, there is usually high levels of interspecific aggression if sympatric congeners have an overlap in their recognition signals (morphological features used as a species identifier). A pronounced example of recognition signal overlap causing heterospecific aggression can be observed in *Hetaerina spp*., a clade of damselflies that are native to the Americas. The mature males of several closely related species in this clade have clear wings with a ruby-red spot at the base of the wing where it attaches to the thorax. Extensive experiments carried out by Grether et al. (2015)[2] showed that it is the ruby-red spot, not other morphological features, that stimulate an aggressive response towards any territory intruders. Since several species share the ruby-red spot feature, when a heterospecific encroaches on an individual's territory, the territory holder cannot distinguish between a competing conspecific or harmless heterospecific so initiate costly aggressive combat[2]. Generally, physical combat is undesirable because it is energetically costly (thus requiring a greater amount of time to be allocated to feeding rather than other important activities such as mating) and could lead to long-term anatomical damage which reduces overall fitness. The cost of combat stimulates agonistic character displacement (evolved characteristics that reduce interspecific aggression). Agonistic character displacement has occurred in one species within this group of damselflies, *Hetaerina titia*. In the late breeding season, dark melanin masks the ruby-red spot of *H.titia* which means that other sympatric congeners can clearly distinguish them as non-threatening and do not initiate an aggressive attack. The question remains, if having darkened melanic wings eliminates costly interspecific aggression, why are *H.titia* wings not permanently in the melanic state?

Introduction: Observations of *H. titia* show that the species exhibits seasonal polyphenism – in the early breeding season most males have clear wings with the ruby-red spot but later in the season the population is almost ubiquitously in the darker, melanic state. This project forms part of a larger body of research investigating the evolutionary causes of this seasonal polyphenism. One hypothesis[3] suggests that the polyphenism in *H. titia* is an evolved response to the seasonal variation in the trade-off between reduced interspecific interference and the risk of being more conspicuous to avian predators[4]. In short, the cost of predation outweighs the possible benefits of reducing interspecific aggression in the early season. Interestingly, only populations on the Atlantic coast of Costa Rica show this meticulous adjustment in phenotype suggesting that it is an evolved response to the combination of interactions within the ecosystem of the Atlantic population. The broader scope of the research project carried out by Dr Jonathan Drury and his colleagues aims to add to and strengthen the evidence that there is indeed a link between seasonal polyphenism and predation by directly measuring the predation risk faced by different *H.titia* populations. I propose to investigate a key factor that is shaped by predation risk – anti-predator behaviour. If anti-predator behaviour evolves in relation to predation risk, we would expect to observe melanic individuals showing greater risk-aversion than non-melanic individuals under the trade-off hypothesis.

Proposed methods: Flight initiation experiments will be used to test whether the anti-predator response of melanic individuals differs from non-melanic conspecifics across several populations in Costa Rica. Flight-initiation experiments involve an investigator slowly approaching a perched damselfly and measuring the distance at which the damselfly flees[5]. Hypothetically, if melanic individuals initiate their escape from an approaching investigator prematurely relative to non-melanic individuals this would suggest that there is a greater perceived predation risk of the more conspicuous melanic morph. The outcomes of this experiment could be used in collaboration with data on the migratory patterns of avian predators – if melanic individuals are under greater threat of predation then the cycle of seasonal polyphenism could have evolved to correspond to with the migratory patterns of the avian predators.

Summary: This project aims to contribute to a growing body of research investigating the evolutionary causes of seasonal polyphenism in the wing colouration of *H. titia* through conducting experiments to test hypotheses about the relationship between melanic individuals and increased predation risk.

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