# The flight initiation distance of Smoky rubyspot damselflies *(Hetaerina titia)* is affected by the abiotic environmental conditions

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# 1 Introduction

Selection favors behaviours that aid the evasion of predation in prey organisms, hence, a vast array of anti-predator behaviours have evolved. These range from distracting displays to passive cryptic colouration [1]. A common anti-predator behaviour in aerial animals is flight initiation. Upon the acknowledgement or approach of a predator, organisms initiate an escape flight to avoid capture [1]. The flight initiation distance (FID) is the distance between the predator and the prey when the prey organism initiates their escape flight. The FID of an organism is an optimized distance that dynamically varies depending on the specific predator-prey scenario; flight is only initiated when the cost of remaining is greater than the cost of leaving (i.e. abandoning a good mating territory) [2]. The cost of remaining or rather the perceived predation risk, could be dependent on a combination of several abiotic, biotic and situational factors. For example, energy is expended on maintaining observations on a distant potential predator so the potential benefits of continuing with normal activity are reduced; FID is larger when the predator starting distance is larger [2]. Another example is how conspicuous the individual is to the predator. More conspicuous predators are likely to be the easier target prey so selection favors a longer FID that mitigates the cost of the conspicuous phenotype.

*Hetaerina spp.*, are 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 brownish-red spot at the base of the wing where it attaches to the thorax. Since several species share the brownish-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 attacks [3]. The cost of combat stimulates agonistic character displacement.

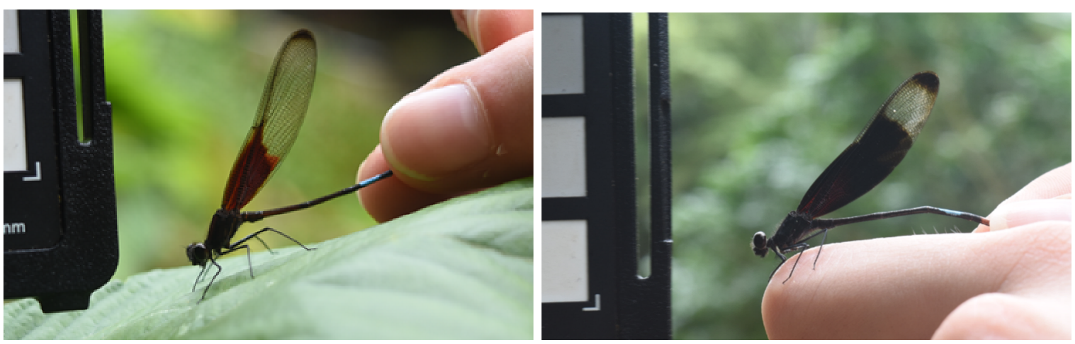

Figure 1: (Left) A mature male *H. titia* from the Golfito district of Costa Rica in the peak breeding season. This individual has clear wings with a brownish-red spot at the base of the wings and a diffuse brown patch at the tip of the wings. (Right) A mature male *H. titia* from the Limón province of Costa Rica in the peak breeding season. This individual has dark pigment masking the brownish-red spot and a dark brown tip.

Agonistic character displacement has occurred in one species within this clade of damselflies on the Atlantic slope of Costa Rica, *Hetaerina titia.* In the peak breeding season, dark melanin masks the brownish-red spot of *H. titia* which means that other sympatric congeners can clearly distinguish them as a non-threat and do not initiate aggressive attacks. However, in the early breeding season the wings of *H. titia* resemble those of their sympatric congeners [4]. It has been proposed that this seasonal 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. In short, the cost of predation could outweigh the possible benefits of reducing interspecific aggression in the early breeding season on the Atlantic slope.

This study investigated whether the melanic morph of *H. titia* is objectively more conspicuous than the common morph (clear wings with a brownish-red spot at the thorax). We hypothesized that objectively more conspicuous individuals on the Atlantic slope would have a larger FID. This hypothesis is based upon the assumption that melanic individuals show greater risk-aversion than non-melanic individuals under the trade-off hypothesis due to them being more conspicuous to predators. We did not find a relationship between conspicuousness and FID, however, we discovered that the microhabitat of a perched *H. titia* is an important factor affecting FID.

Specifically, we noticed a strong relationship between the gap fraction (GF) and FID. Gap fraction describes the proportion of the canopy cover above the perch which is open sky rather than foliage and trees. Perhaps individuals inhabiting areas with less dense canopy cover have earlier detection of predation risk than individuals under a dense, closed canopy because the environmental light intensity is greater, and it is easier to visually detect an incoming predator. Alternatively, these better light conditions may provide more frequent opportunities for avian predators to spot the damselflies, so the damselfly learns to evade predators earlier to mitigate the high predation risk. Furthermore, energetically costly anti-predator behaviours such as flight initiation are largely dependent on temperature [5]. Therefore, habitats with open canopy covers provide greater insolation to increase the body temperature of the damselflies meaning the energetic cost of leaving is lower and flight can be initiated sooner.

# 2 Methodology

## 2.1 Experimental Procedure

In this study we conducted flight initiation distance (FID) experiments on male *H. titia* damselflies exclusively. In the Golfito district on the Pacific slope of Costa Rica, we carried out (n=60) trials of our FID experiment across five sites between the 06/24/22 and 07/01/22. In the Limón province on the Atlantic slope of Costa Rica, we carried out (n=47) trials of our FID experiment across three sites between the 07/11/22 and 07/12/22.

Figure 2: A map of Costa Rica showing the exact locations of each site we conducted FID experiments.

This experiment was carried out by two investigators wearing neutral coloured clothing. Two investigators waded through the centre of a stretch of river at each site and used binoculars to scan the river bank for *H. titia* males perched at territorial height (<1.0m above river surface). Once the focal individual was identified as *H. titia* we took a photograph of the side profile of the damselfly (Panasonic Lumix DC-FZ82 with x60 optical zoom) from a distance of at least 5m away, as not to startle the individual.

The air temperature of the site was take approximately every hour. The starting point of an approaching investigator (a proxy for an incoming predator) was clearly marked. Then the approaching investigator would stride towards the focal damselfly at a pace of approximately 1m/s and immediately stopped once the focal damselfly initiated their escape flight. The stopping point of the investigator was clearly marked and, when possible, the re-perch position of the focal damselfly was noted. The second investigator would then use a level tape measure to measure the FID and starting distance (see figure 3).

Finally, to quantify the canopy cover over the initial perch of the focal damselfly, we used the x0.5 zoom function on the front-facing camera of an iPhone to take a skyward photo at the same height and location as the initial perch position. The cloud coverage was noted and assigned the number 1 ($\leq$ 50% cloud coverage) or 2 ($>$ 50% cloud coverage or shaded perch).

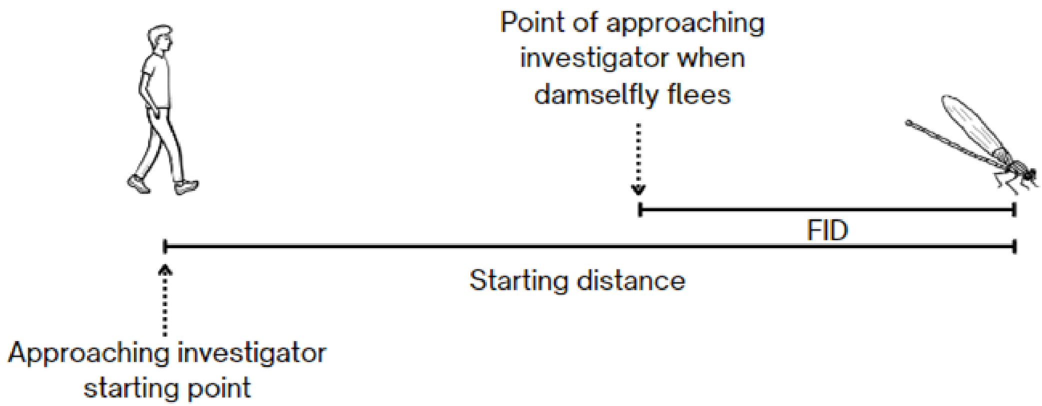


Figure 3: A diagram indicating how we defined FID and starting distance in our experiment. The flight initiation distance is the distance between the approaching investigator at the time of the focal damselfly's escape and the damselfly's initial perch. The starting distance is the distance between the starting point of the approaching investigator and the focal damselfly's initial perch.

To ensure that the experiment was carried out on an individual only once, we walked at least 10m away from the previous experiment site before scanning the river again for a new damselfly.

## 2.2 Conspicuousness

In this study we define and quantify conspicuousness as the In-transformed greyscale value of the background surrounding an individual minus the In-transformed greyscale value of the entire wing of an individual. All images were analyzed using the software package ImageJ [6].

## 2.3 Gap Fraction (GF)

The digital cover photographs were analyzed using an R package, *coveR*, which enabled us to obtain objective measurements of the proportion of foliage present in the digital cover photographs [7]. One of the measurements calculated by *coveR* is gap fraction (GF). This variable describes the what proportion of the canopy cover above the perch is open sky.

## 2.4 Statistical approach

In our analysis, we included the following variables as predictor variables for our response variable (FID): the slope the FID trial was carried out on, starting distance, temperature, proportion of wing covered by black pigment, conspicuousness, cloud coverage at the time of the experiment, and the gap fraction (GF) of the canopy cover directly above the perch of the individual.

The standard set of variables included in all six models were slope, starting distance and temperature. In $M_1$ we also included the proportion of black pigment index. In $M_2$ we included conspicuousness instead of the black pigment index. The proportion of black pigment index and conspicuousness are two highly correlated variables therefore to disentangle their potential effect on FID we separated the two variables into different models. $M_3$ and $M_4$ contained the same variables as $M_1$ and $M_2$ respectively, however, we also included GF in $M_3$ and $M_4$. The rationale for excluding GF from $M_1$ and $M_2$ was that some of the digital cover photos were poorly analyzed by the *coveR* package therefore they had to be excluded from our analysis, reducing the sample size. Finally, $M_5$ and $M_6$ contained the same variables as $M_1$ and $M_2$ respectively but cloud coverage was also included.

# 3 Results

## 3.1 FID and Wing pigmentation

All six of the linear regression models show that there is no significant correlation between wing pigmentation and FID ($P > 0.05$). Therefore, we can infer that the proportion of wing covered with black pigment and the conspicuousness of an individual are unlikely to affect the FID of the perched individual.

However, as shown by figure 4, there is a large difference in the conspicuousness of individuals between the Pacific and Atlantic slope populations. The mean conspicuousness value for individuals on the Atlantic slope is greater than individuals on the Pacific slope ($1.385 > 0.563$). An independent t-test determined that there is a statistically significant difference in the mean conspicuousness value between the Pacific and Atlantic slopes ($t_{102} = 9.448, p = 1.359 \times 10^{-15}$).

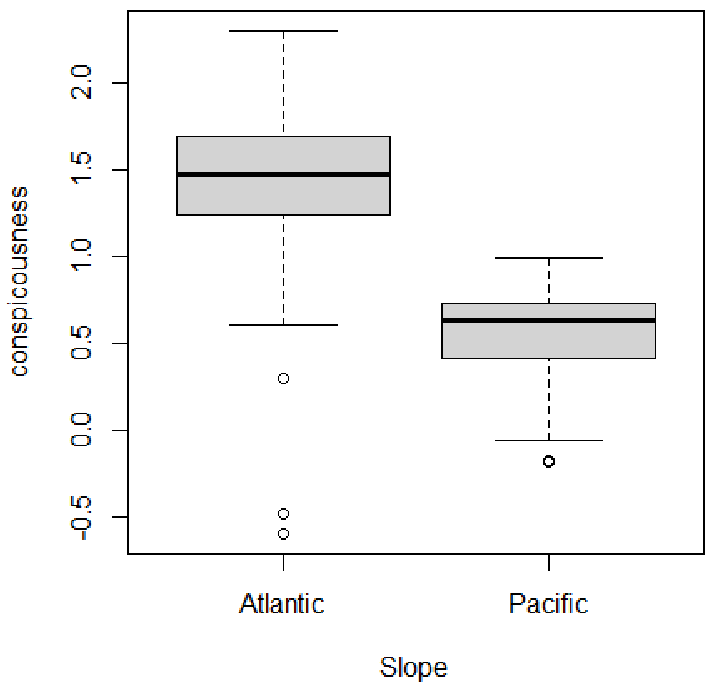


Figure 4: A boxplot showing the difference in the median conspicuousness value between the Atlantic and Pacific slope of Costa Rica.

## 3.2 FID and Start Distance

Five of the linear regression models suggest that there is a positive correlation between starting distance and FID (See Table 1). This means that it likely that the starting distance of the approaching investigator impacts the FID of the perched individual. A larger start distance is linked to a larger FID. This is an expected relationship; energy is expended on maintaining observations on a distant potential predator so the potential benefits of continuing with normal activity are reduced, consequently, FID is larger when the predator starting distance is larger[2].

## 3.3 FID and Temperature

All of the linear regression models suggest that there is no relationship between FID and temperature ($P > 0.05$). Therefore, we can conclude that temperature did not affect FID.

## 3.4 FID and GF

$M_3$ and $M_4$ show that there is a strong positive correlation between GF and FID (See Table 1). This means that it is highly likely the canopy cover over the initial perch location affects the FID of an individual. When there was a greater proportion of open sky relative to canopy cover over the perched individual, the FID was larger.

An independent t-test determined that there is a statistically significant difference in the mean GF value between the Pacific and Atlantic slopes ($t_{54.8} = 2.862$, p = 0.005). The mean GF value for the Atlantic slope was greater than the Pacific slope (0.456 > 0.290).

### 3.5 FID and Cloud cover

$M_5$ and $M_6$ show that there is no correlation between cloud coverage and FID. However, in this investigation there was a strong link between GF and Cloud cover. Experimental trials where there was a high GF value often had low cloud coverage. These two variables are not dependent on one another but rather cloud coverage on the Pacific slope was always $\geq 50\%$ during our experiments and the mean GF was lower. This makes it difficult to disentangle the effect of these two abiotic conditions on FID.

Table 1: A table presenting the estimated R value, Standard error and P-value for each variable in each of the six linear regression models (1-6).

| Variable | Estimate R value | Std. Error | P-value |
|---|---|---|---|
| Start distance | 0.304 | 0.122 | 0.014 |
| Temperature | -0.069 | 0.122 | 0.394 |
| Prop. of black index | -1.647 | 0.079 | 0.072 |
| Slope (Pacific) | -0.726 | 0.360 | 0.046 |
| Start distance | 0.353 | 0.125 | 0.006 |
| Temperature | -0.111 | 0.083 | 0.187 |
| Conspicuousness | -0.080 | 0.170 | 0.637 |
| Slope (Pacific) | -0.153 | 0.207 | 0.460 |
| Start distance | 0.221 | 0.131 | 0.095 |
| Temperature | -0.0069 | 0.087 | 0.435 |
| Prop. of black index | -1.900 | 0.986 | 0.057 |
| Slope (Pacific) | -0.822 | 0.398 | 0.042 |
| GF | 0.719 | 0.314 | 0.025 |
| Start distance | 0.293 | 0.132 | 0.029 |
| Temperature | 0.293 | 0.132 | 0.029 |
| Conspicuousness | -0.394 | 0.206 | 0.060 |
| Slope (Pacific) | -0.351 | 0.233 | 0.135 |
| GF | 0.998 | 0.342 | 0.004 |
| Start distance | 0.279 | 0.121 | 0.0235 |
| Temperature | -0.013 | 0.084 | 0.875 |
| Prop. of black index | -1.601 | 0.898 | 0.078 |
| Slope (Pacific) | -0.489 | 0.378 | 0.199 |
| Cloud Prop. | -0.421 | 0.226 | 0.066 |
| Start distance | 0.324 | 0.121 | 0.011 |
| Temperature | -0.056 | 0.0887 | 0.531 |
| Conspicuousness | -0.114 | 0.169 | 0.503 |
| Slope (Pacific) | 0.039 | 0.233 | 0.866 |
| Cloud Prop. | -0.415 | 0.240 | 0.087 |

## 4 Discussion

This study shows that conditions created by canopy cover affect the anti-predator behaviour of smoky rubyspot damselflies (*H. titia*) in Costa Rica. In ravine habitats where the canopy cover is less dense and there is more open sky directly above the perch of individuals FID is longer (see Figure 5). One hypothesis that could explain our observation is that the light intensity at territorial perch height in open canopy cover habitats is greater than in closed canopy cover habitats therefore individuals can visually detect the threat of an incoming predator earlier and consequently initiate their escape earlier. Some may argue that, alternatively, open canopy cover habitats provide avian predators with greater light intensity and an improved ability to identify prey targets. Therefore, there are more frequent predation attempts and predation risk is higher. Potentially, the damselflies may learn to initiate their escape flight earlier in order to mitigate this increased predation risk.

Figure 5: An example of a site on the Atlantic slope. The territorial perches along the river at this site were exposed to the full sun.

Although we did not identify a relationship between cloud cover and FID, we still cannot rule out the possibility of cloud cover affecting FID. On the Pacific slope of Costa Rica there were zero days of low cloud coverage whilst we were conducting experiments and mean GF value was significantly lower than on the Atlantic coast. This makes it difficult to disentangle these two abiotic variables - we cannot be certain that it was the low GF that was affecting FID if the cloud coverage (a variable that could also affect light intensity) was always high.

To further extend this new enquiry into abiotic conditions affecting FID in *H. titia*, a greater sample size is needed. Whilst controlling for other abiotic variables, experimental trials need to be conducted in several habitats with a range of canopy cover extent on both slopes of Costa Rica. This would confirm that canopy cover affects FID, not another variable that is dependent on the slope the experiment is carried out on. Additionally, experimental trials would need to be carried out in a range of cloud cover conditions in both low and high GF habitats. This would also us to disentangle the effects of GF and cloud cover on FID.

If abiotic conditions significantly affect the anti-predator behaviour of *H. titia*, it should be considered that drastic changes to the abiotic features of the rainforests and ravine habitats of Costa Rica could affect the levels of predation and, consequently, the population of this species. The ongoing climate crisis and shift to agricultural plantations rather than rainforests could lead to changes in the cloud cover and canopy cover over the habitats of *H. titia*. Furthermore, studies have shown that organisms adapt their fear behaviour in order to maintain their fitness in environments where there is high levels of disturbance [8].Therefore, the anti-predator behaviour of *H. titia* in ravine habitats surrounded by urban land may also be affected.

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