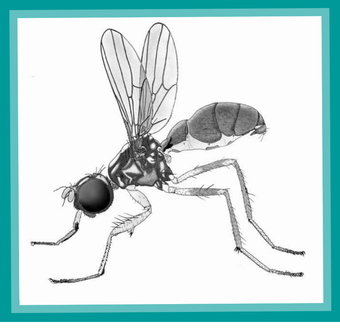

Investigating species limits and sexual selection in the "widespread" *Allosepsis indica*

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Introduction

Allosepsis indica is a "widespread" species of the dipteran family Sepsidae. It is easily recognised based on two protrusions on the male fore leg femur, one of which bears four thick spines (Fig. 3). However, a previous study demonstrates that the mitochondrial *COI* gene sequences are extremely divergent across populations (≤13.8%), which suggest the presence of multiple species.

The mating behaviour of *A. indica* is also peculiar. In trials, the mating success rates of virgins are remarkably high (90%) despite the lack of courtship behaviour. This lack is uncharacteristic for Sepsidae. Females also offer no resistance to male attempts during their first matings but apparently refuse subsequent matings fervently; i.e., the species may be monogamous.

Objectives

1. To determine the species boundaries through comparative analyses of **genetic**, **morphological** and **behavioural** data.
2. To provide a comprehensive mating profile for *A. indica* through extensive mate trials and recordings.
   - Investigate evidence for or against female-enforced monogamy
   - Understand the high success rate of mating trials with virgins
   - Understand why males have elaborate male fore leg armature despite the lack of female resistance

Materials and Methods

1. Genetic Analysis

Genomic DNA Extracted → *COI* and *Cyt B* Amplification & Sequencing → Sequences Aligned → Phylogenetic Tree; Clustered based on genetic distances using Species Identifier

2. Morphological Analysis

Genetically distinct specimens scrutinised for character differences → Characters are examined for all other specimens, under high magnification.

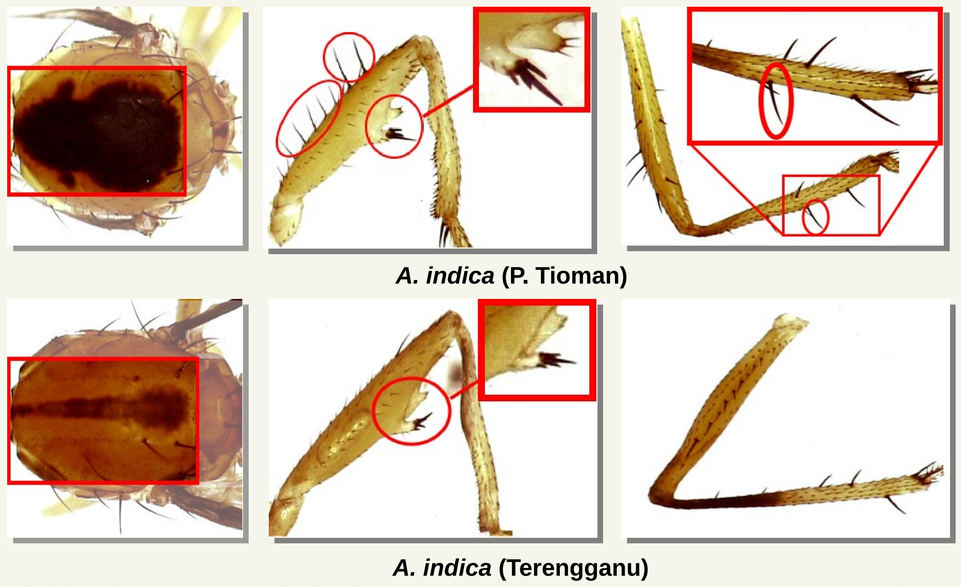
Figure 1. *A. indica* in copulation

3. Behavioural Analysis

New Emergents → Virgin ♂ / Virgin ♀ → Mate Trials → Analog Recordings → Digitization, Frame-by-frame analysis → Mating Profile

Morphological Analysis

- Genetically diverse populations differ only by the dorsal thorax pigmentation, shape of the blade on the fore femur and number of bristles on the legs (Fig. 3)
- However, upon inspection of all populations with genetic data, these characters are continuous **(157 specimens)** and do not allow for recognising multiple species based solely on morphology



***A. indica* (P. Tioman)**

***A. indica* (Terengganu)**

Figure 3. Morphological differences that exist between genetically diverse P. Tioman and Terengganu populations

Reproductive Isolation Analysis

2 populations were tested. The maximum pairwise distance between P. Tioman and Terengganu populations were

15.9% (*COI*) & 12.3% (*Cyt B*)

A. indica from P. Tioman and P. Terengganu are reproductively isolated

- High mating success for virgin matings observed for mate trials using Terengganu flies (**61.1%**)
- However, in hybridization experiments females show no interest and shake off males from other populations
- Reproductive isolation is likely to be driven by differences in mating behaviour :

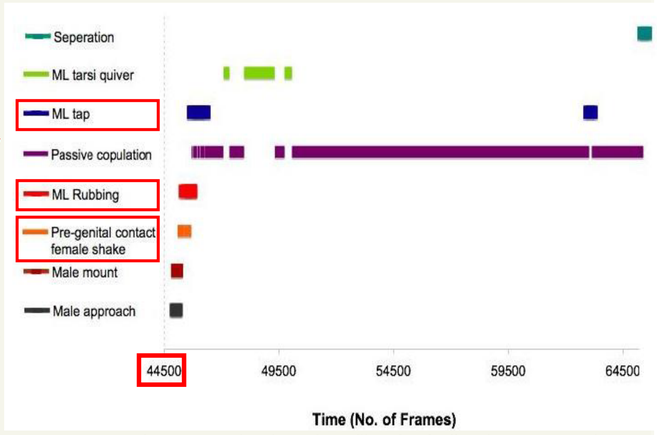


Figure 4. Behavioural profile of *A.indica* (Terengganu)

	Degree of Female Resistance (1st Trial)	Male Response	Copulation Behaviour	Time Lapse before Male Mount	Length of Copulation
P. Tioman	No Resistance	-------	------	Almost immediate	~ 7mins
Terengganu	Body Shake	Mid leg (ML) Rubbing	ML Tap	30 - 40 mins	~12 mins

Table 1. Aspects of mating behaviour that differ between the 2 populations

Figure 5. Before and after pictures depicting mid leg (ML) tap

Genetic Diversity within *A. indica*

- *COI* and *Cyt B* sequencing was successful for 27 Specimens (50 sequences - Coded **blue** in Fig. 2)
- Maximum pairwise distance for

  COI : **16.2 %** (Between Terengganu & Kuala Lumpur)

  Cyt B: **16.1%** (Between Frazers Hill & P. Tioman C)

- The additional sequences reinforce the finding of high genetic diversity from previous studies
  - Confirms high genetic variation between populations
  - **6** genetic clusters were produced through the clustering of sequences that were 3 - 6% apart (See **orange** boxes in Fig. 2)

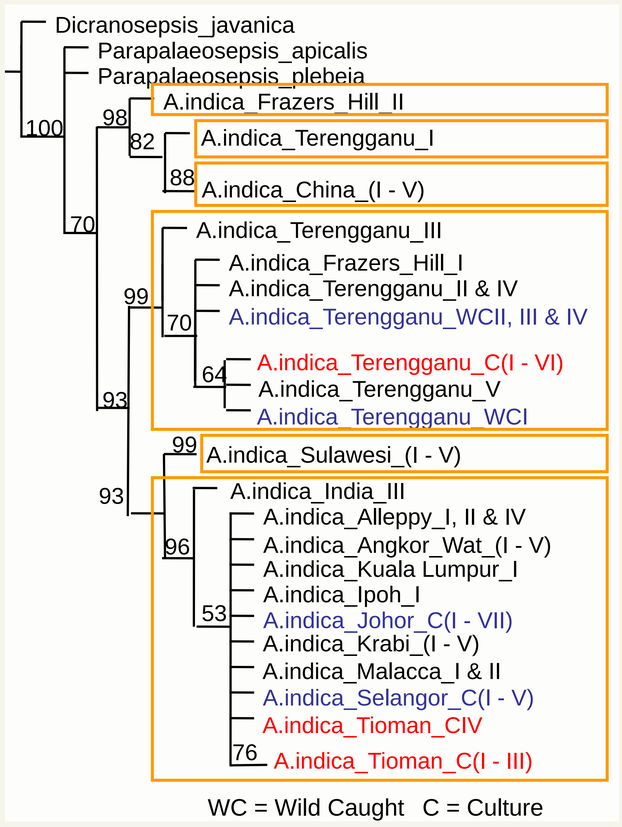


Figure 2. Bootstrap tree based on *COI* and *Cyt B* Sequences

Sexual selection within *A. indica*

Is *A. indica* monogamous?

- In contrast to the earlier suggestions, successful rematings were observed albeit at much lower rates than during trials with virgins (Fig. 6)
- Very drastic change in observed female response to male attempts

1st Trial - No female resistance

Remating - Violent body shake, Leg raise, Head butt, Chasing

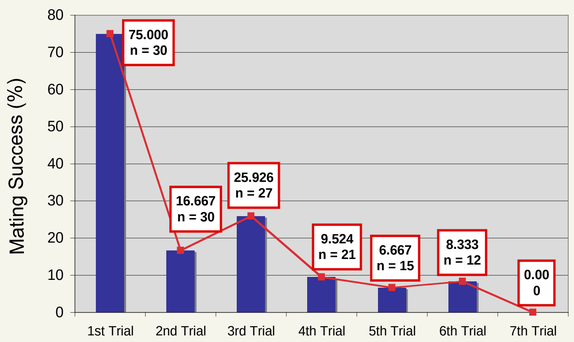


Figure 6. Graph showing mating success in first and subsequent trials

Why is mating success so high in virgin trials?

- Unequal sex ratio among new emergents (Fig. 7) might explain why it benefits virgin females to mate readily with the first male she encounters
- Hence, the female resistance is only observed in remating trials

What determines mating success in 'remating' trials?

- There is no apparent size advantage in males

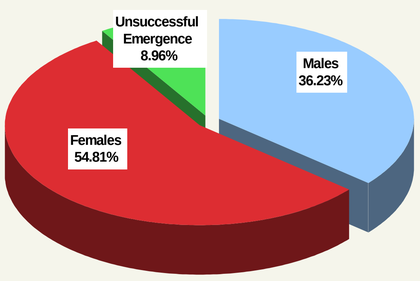


Figure 7. Graph showing sex distribution of new emergence including proportion of fertilised eggs that failed to produce adults

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