

***Maevia inclemens* (Araneae: Salticidae: Marpissina) copulation duration is longer with alternate morph males**

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Abstract. The polyandrous Dimorphic Jumping Spider, *Maevia inclemens* (Walckenaer 1937), maintains a 1:1 ratio of the two male morphs in all natural populations investigated. Hill (2023) provided a hypothesis and mathematical model for how this 1:1 ratio might be maintained in populations: mainly, through female preference for the alternate morph after initial mating. We present laboratory results that support Hill's model for stabilizing selection in *Maevia inclemens*. Additionally, we find that the preference of *M. inclemens* is specific to novel morphs, not just novel males.

Keywords. dimorphic males, female choice, jumping spider, spider behavior

Introduction

The Dimorphic Jumping Spider, *Maevia inclemens* (Walckenaer 1937), is best known for having two distinct male morphs (Painter 1913). The two male morphs differ in appearance and courtship behavior: the gray morph has coloration similar to the female and the tufted morph has a black body with three tufts of black setae medially just in front of the posterior eye row (see Hill 2023 for photos of the female and male morphs as well as courtship behavior).

At present, *Maevia* is the only known salticid genus with dimorphic males, with two described species (*M. inclemens* and *M. expansa* Barnes 1955) and one undescribed species having distinct morphs (G. B. Edwards, personal communication 2022).

For *Maevia inclemens*, an approximately 1:1 ratio of male morphs has consistently been observed in field populations (Clark 1992; Lietzenmayer & Taylor 2018), but the mechanism for the maintenance of this stable 1:1 ratio is unknown. Field observations have failed to find any significant difference between the morphs in habitat, microenvironment, or predation/parasitism (Clark 1992; Pascoe unpublished).

However, Hill (2023) described a working hypothesis for the maintenance of a stable 1:1 ratio of male morphs in *M. inclemens*. His hypothesis has three requirements:

First: Females must mate multiple times over a period of at least several days.

Second: Females must prefer matings with alternate morphs (i.e., different morph than the previous mating)

Third: Either morph must have a similar likelihood of mating an unmated female.

Hill concluded if the three statements above are confirmed, then, as one morph becomes more common, more virgin females will encounter and mate with it. These mated females will then prefer the less common morph for subsequent matings, which will increase the frequency of the less common morph. Thus, the stable 1:1 ratio is maintained. Hill supplements his hypothesis with a mathematical model for maintaining two morphs in a population.

The first and third requirements for Hill's hypothesis are already supported with published field observations and laboratory studies:

First: Laboratory studies have documented female polyandry in *M. inclemens* (Painter 1913; Clark & Biesiadecki 2002). Indirect evidence for polyandry in the field comes from comparisons of females mated to only one morph in the laboratory (which produced almost a 2:1 ratio in favor of the morph used for mating) vs. field collected females (which produced 1:1 morph ratio) (Clark 1992).

Third: Unmated females' lack of preference for one morph over the other has also been documented (Clark & Uetz 1990, 1992, 1993; Clark & Morjan 2001; Clark & Biesiadecki 2002)

Here we investigate Hill's second requirement (that females prefer to mate with alternate male morphs) through laboratory experimentation. Beyond a female preference for alternate male morphs, we address female preference for any "novel" male, including a preference for a different male of the same morph. Thus, this novel male hypothesis includes the prediction that females will prefer any different male from her previous mating, not just alternate morph males.

Because virgin females showed no significant difference between male morphs in mean copulation duration when mated to a single male (Clark & Biesiadecki 2002), we use difference in copulation duration with subsequent males as an indicator of female preference. Unlike other salticid matings, *Maevia inclemens* females do not always "freeze" and remain stationary during copulation. There often is significant female resistance during mating, including walking and "leg wrestling." Therefore, we distinguish two separate parts of copulation: mount and palp insertion. The "mount" is defined as the time elapsed from initial male contact with the female and until contact is terminated. Mounts are often preceded by one or more male pounces (jumps toward or onto the female from variable distances). Not all mounts resulted in the male successfully inserting one or both palps into the female genitalia for semen transfer. "Palp insertion" is defined as time in which male palps are inserted into female genitalia for semen transfer. Since previous studies did not specify if "copulation" referred to one or both aspects, we measured duration of both mount and palp insertion.

If, after the first mating, female preference is for a novel male morph (Hill's hypothesis), we predicted the relative duration for both mount and palp insertion (for second and subsequent matings) to be as follows:

$$\boxed{\text{novel male of the alternate morph}} > \boxed{\text{novel male of same morph}} > \boxed{\text{same individual}}$$

If, after the first mating, female preference is for any novel male (regardless of morph), we predicted the relative duration for both mount and palp insertion (for second and subsequent matings) to be as follows:

$$\boxed{\text{novel male of the alternate morph}} = \boxed{\text{novel male of same morph}} > \boxed{\text{same individual}}$$

Methods

Adult and penultimate *M. inclemens* specimens were collected by hand and sweep net in late spring/early summer at several field sites in Northeastern Illinois (Will County). The spiders were housed separately in one-pint opaque circular deli containers with a moist cotton wad to maintain humidity. They were fed wingless fruit flies (*Drosophila*) daily.

The mating preference trials were performed in a laboratory at room temperature (21°C). The experimental chambers were open-topped, plastic containers (22 cm length X 12 cm width) with additional incandescent lighting. The inside walls were covered with a thin layer of petroleum jelly above 2 cm to prevent spiders from leaving the chambers. For each trial a male and a female were introduced on opposite sides of the containers and observed for 10 minutes. Only the first copulation of any 10-minute trial was used to eliminate any artifact of the spiders being unnaturally confined in the experimental chamber. Male trials were grouped into one of four treatments with a female:

treatment		description	trials (n)
FM	<i>FirstMate</i>	the first male the female was introduced to	25
SI	<i>SameIndividual</i>	the female was introduced to the same individual male as the previous trial	10
NS	<i>NovelSame</i> : Novel male of Same morph	the female was introduced to a different individual male from the previous trial but of the same morph	33
NA	<i>NovelAlternate</i> : Novel male of Alternative morph	the female was introduced to an alternate male morph from the previous trial	22

Trials were 20-30 minutes (or more) apart. A single female was used for several trials/day. All trials were videotaped with a Canon XL1S MiniDV Camcorder. Recordings were watched and the following behaviors were measured as indicators of female preference for males in different treatments and males of different morphs:

Duration of mount (including duration of palp insertion)

Duration of palp insertion

Time to mount from female orientation to male

Number of male pounces preceding mount (counted as any time the male jumped or attempted to jump on the female).

All statistical analyses were done in R (version 3.6.0). An alpha value of 0.05 was used to determine significance for all statistical testing.

To determine differences in mating behaviors by treatment, we ran generalized linear mixed effects models using the R package “lme4” version 1.1—27.1 (Bates et al. 2015) with a Poisson distribution. We tested the variables (duration of mount (secs), duration of palp insertion (secs), time to copulation after orientation to male (secs), and number of pounces performed by the male) as a function of treatment with female identification number as a random effect. To account for overdispersion of the data, we add in an observation-level random effect (Harrison 2014). We conducted hypothesis testing on the models using ANOVA tests in the “car” package (version 3.1–1) (Fox & Weisberg 2019). Post-hoc pairwise comparisons were made using estimated least squares means (package “emmeans” version 1.7.0, Lenth et al. 2023) to determine differences between pairs of treatments.

To determine differences in mating behaviors by morph within First Mate trials, we ran generalized linear mixed effects models (package “lme4” version 1.1—27.1) (Bates et al. 2015) with a Poisson distribution. We tested the variables (duration of mount (secs), duration of palp insertion (secs), time to copulation after orientation to male (secs), and number of pounces performed by the male) as a function of male morph with female identification number as a random effect. To account for overdispersion of the data, we add in an observation-level random effect (Harrison 2014). We conducted hypothesis testing on the models using ANOVA tests in the “car” package (version 3.1-1) (Fox & Weisberg 2018).

Results

All females copulated with more than one male (although not always on the same day) and often copulated (mount with or without palp insertion) more than once with the same male during a 10 min trial. Only first copulations for a trial are used in this study. While males often pounced on females and sometimes wrestled with them before mounting, females appeared able to end a mount or palp insertion by shrugging males off, usually after a few seconds. After this, the female usually immediately attempted to flee and escape from the experimental container. Approximately 8% of mounts ended with no palp insertion. The female’s ability to end a mount was supported by observations of a male mounting an immobile female and inserting one palp after another for several minutes – much longer than the typical few seconds mount/palp insertion observed with active females. See Appendix 1 for data.

Duration of mount: Duration of mount differed by treatment ($X^2=14.078$, $df=3$, $p=0.0028$); NovelAlternate (NA) males had significantly longer mounts than NovelSame (NS) males ($p=0.0059$) and SameIndividual (SI) males ($p=0.0196$) (Figure 1).

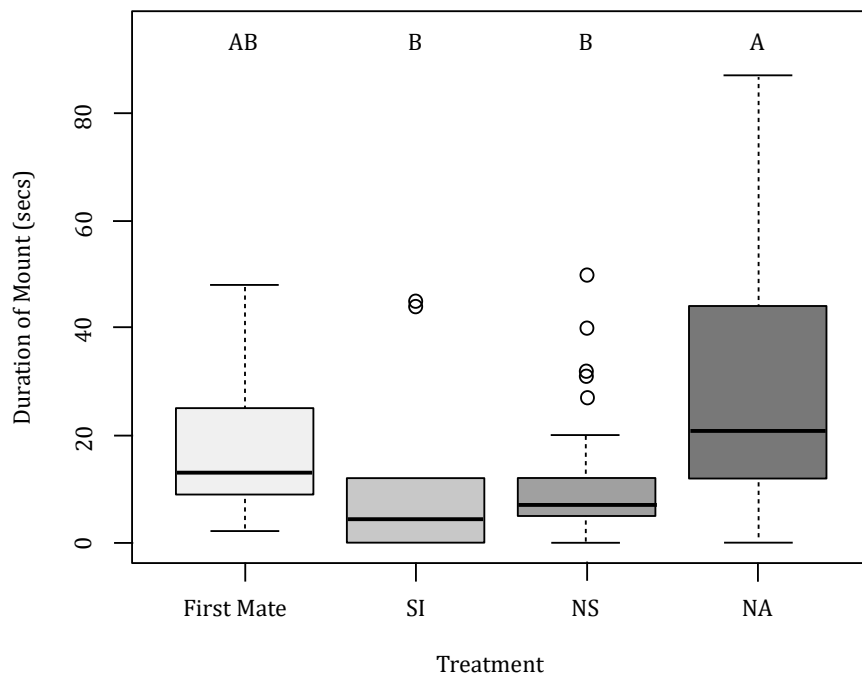


Figure 1. Duration (secs) of mount. Boxes represent the upper and lower quartiles. Lines in boxes represent medians. Duration of mount differed by treatment ($X^2=14.078$, $df=3$, $p=0.0028$). Letters above boxes indicate significant differences. Treatments that share a letter do not differ in duration of first mount. NovelAlternate (NA) males had significantly longer mounts than NovelSame (NS) ($p=0.0059$) and SameIndividual (SI) ($p=0.0196$). In Figures 1-5, whiskers represent the minimum and maximum values in the data, except for outliers, which are represented as open circles. Outliers were not excluded from data analysis.

Duration of palp insertion: Duration of palp insertion differed by treatment ($X^2=13.232$, $df=3$, $p=0.004161$); NovelAlternate (NA) had significantly longer palp insertions than NovelSame (p=0.005) and SameIndividual (SI) (p=0.034) (Figure 2).

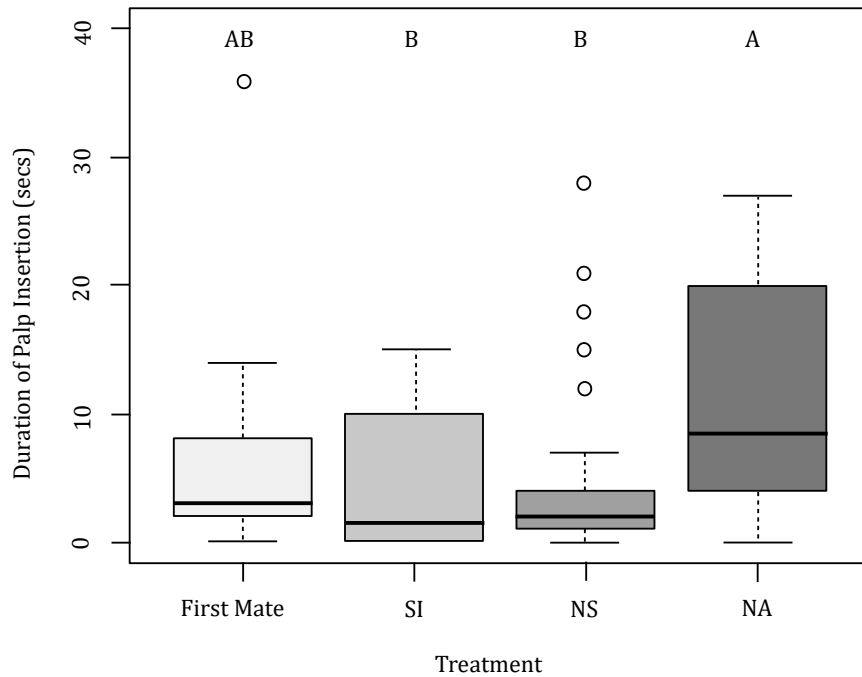


Figure 2. Duration of time (secs) male palps were inserted during mount. Boxes represent the upper and lower quartiles. Lines in boxes represent medians. Duration of palp insertion differed by treatment ($X^2=13.232$, $df=3$, $p=0.004161$). Letters above boxes indicate significant differences. Treatments that share a letter do not differ in duration of palp insertion. NovelAlternate (NA) had significantly longer palp insertions than NovelSame (p=0.005) and SameIndividual (SI) (p=0.034).

Time to mount from female orientation to male: Time to mount after female orientated to the male differed by treatment ($X^2=8.1387$, $df= 3$, $p= 0.04323$). However, post-hoc pairwise testing revealed no significant difference between pairs (Figure 3).

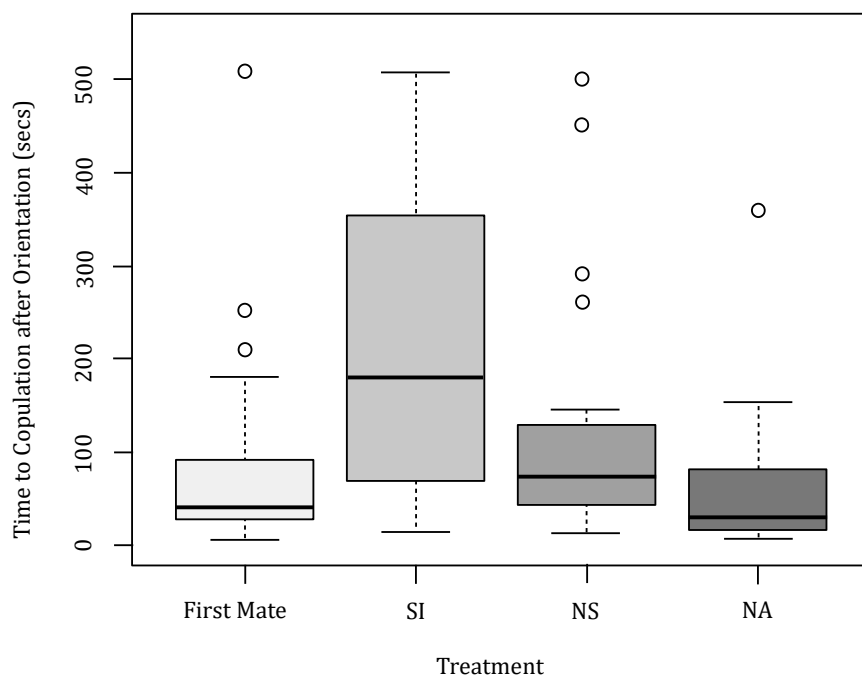


Figure 3. Time (secs) to first mount after female orients to male. Boxes represent the upper and lower quartiles. Lines in boxes represent medians. Time to copulation after orientation differed by treatment ($X^2=8.1387$, $df= 3$, $p= 0.04323$). However, post-hoc pairwise testing revealed no significant difference between pairs.

Number of pounces before mount: The number of pounces before mount did not significantly differ between treatments ($X^2=6.8534$, $df= 3$, $p= 0.07672$) (Figure 4).

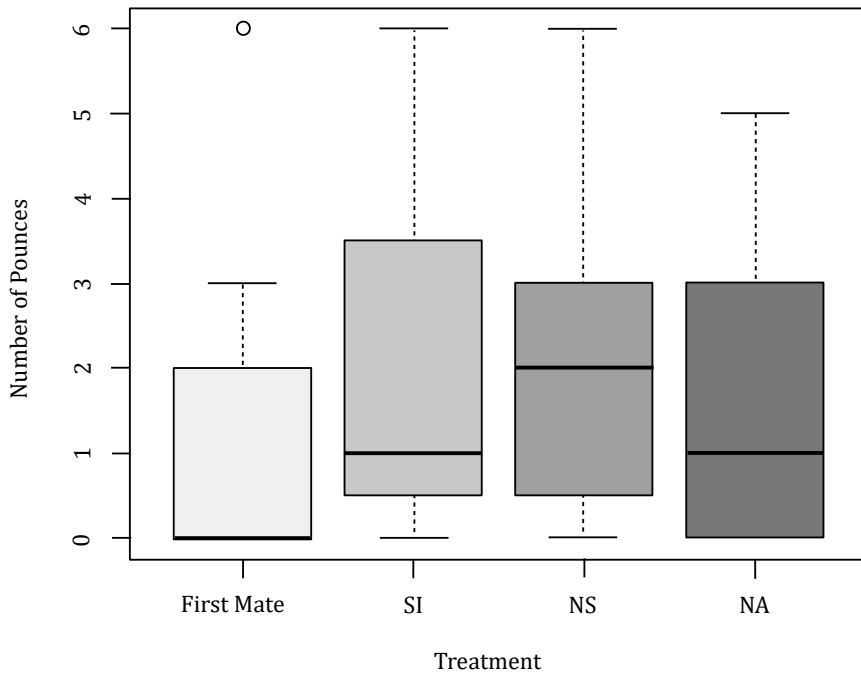


Figure 4. Number of pounces by male before mount. Boxes represent the upper and lower quartiles. Lines in boxes represent medians. Number of pounces by male spiders did not differ by treatment ($X^2=6.8534$, $df=3$, $p= 0.07672$).

Difference between morphs: There were no significant differences between the gray and tufted morph for any of the variables tested: Duration of mount ($X^2=0.0086$, $df=1$, $p=0.9262$), duration of palp insertion ($X^2=0.0549$, $df=1$, $p=0.8147$) (Figure 5), duration of time to copulation after orientation ($X^2=0.6012$, $df= 1$, $p= 0.4381$), number of pounces ($X^2=1.7122$, $df= 1$, $p= 0.1907$). Only FirstMate treatments were used for these calculations.

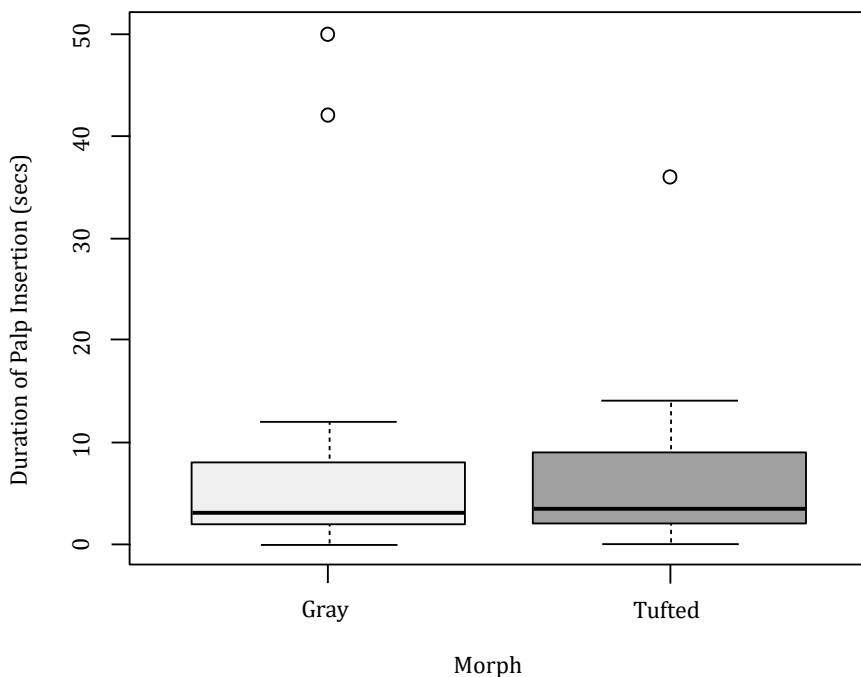


Figure 5. Duration of palp insertion by male morph. Boxes represent the upper and lower quartiles. Lines in boxes represent medians. Duration of palp insertion did not differ by male morph ($X^2=0.0549$, $df=1$, $p=0.8147$).

Discussion

Results for duration of mount, palp insertion, and time elapsed to beginning of mount, all provide support for Hill's (2023) hypothesis. Support for the novel male hypothesis is less convincing. Specifically:

Mount: Hill's second requirement for his model, that mated females must prefer alternate morph males, was met with a significantly longer duration of mount for NA males than either SI or NS males (Figure 1). The novel male hypothesis prediction is not supported with mount duration, as neither NS nor SI had increased mount times, compared to FM. It is worth noting, while not statistically significant, that the median duration for SI and NS mounts was less than that of FM mounts. This supports the conclusion that the SI and NS mounts are less important to the female than either the FM or NA mounts.

Palp insertion: Hill's hypothesis is supported with a significantly longer duration of palp insertion for NA males than SI and NS (Fig. 2). The novel male hypothesis predictions are not supported by these results. Similar to mount duration, the mean duration for FM palp insertion was approximately double that of SI and NS mounts. Palp insertion can be considered the single most important indicator of male mating success and consequence of female preference. A summary diagram (Figure 6) illustrates the comparison between treatments.

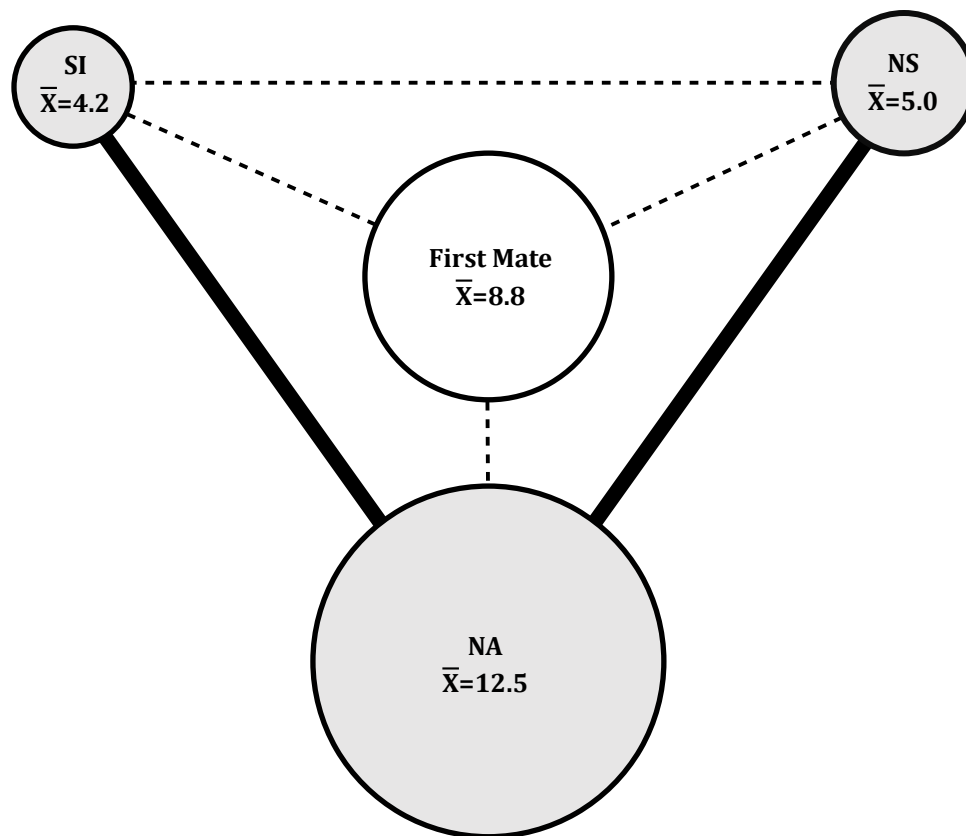


Figure 6. Summary of duration of palp insertion (in seconds) by treatment. The size of circle represents differences in mean palp duration for each treatment. Dashed lines represent no statistical difference between treatments. Heavy lines indicate statistical significance between treatments: NovelAlternate (NA) had significantly longer palp insertions than NovelSame ($p=0.005$) and SameIndividual (SI) ($p=0.034$).

Time to mount from female orientation to male: This measurement was made with the assumption that a shorter time to mount is an indicator of female receptivity and that male aggression does not differ between morphs (see next section). Results do provide some support for both Hill's hypotheses and the novel male hypothesis, as the trend predicted by these hypotheses is seen when comparing medians (Figure 3). The median time to mount was at least double for SI males than either of the novel male treatments.

Pounces: No significant difference in the number of pounces between treatments (Figure 4) suggests no difference in male courtship intensity. This is an interesting result given that courtship behavior is the only behavior controlled by the males and may be another indication that duration of mount and palp insertion are determined by female choice.

Difference between morphs: No significant difference in duration of mount between gray and tufted morphs confirms there is no difference in initial female preference for a morph (Figure 5). This result agrees with previous studies (Clark & Uetz 1990, 1992, 1993; Clark & Morjan 2001; Clark & Biesiadecki 2002).

In the field, *Maevia* are typically found wandering in leaf litter or on flat leaf surfaces (such as Poison Ivy) (Clark 1992; Pascoe unpublished). These habitats allow the females to easily escape males after a copulation compared to the confined containers in laboratory experiments. Field studies of female behavior will help corroborate the behavior observed in laboratory experiments.

While both the maintenance of dimorphic male morphs and polyandrous female's preference for alternate male morphs are apparently unique to *Maevia*, another distinctive aspect of *Maevia* is the short duration of copulation when compared to other salticids. A literature review of copulation duration in 22 species of salticids (Elgar 1995) found a range of 0.7 - 900 minutes, an average of 70.4 minutes, and a median of 11.45 minutes. The copulation duration for *Maevia* recorded in this study and Clark & Biesiadecki (2002) (tufted = 5.6 sec, gray = 5.1 sec) are well below the range of copulation times in all other salticids observed so far. Studies of predation/parasitism, reproductive morphology, preventing male insertion of sperm plug, and cohabitation have been listed as possible influencers of copulation duration in spiders (Elgar 1995) and are worth further study in *Maevia*.

Cannibalism has also been suggested as an influencer of copulation duration (Elgar 1995). While female resistance including "leg wrestling" and female pouncing at males was common in the current study, female cannibalism of a male did not occur (one instance of a male cannibalizing a female was observed). Even in a separate set of mating experiments (Pascoe, unpublished), when females were not fed for 0 - 8 days, cannibalism of a male by a female only occurred 4 times out of 271 trials. Cannibalism of a female by a male only occurred once during the 271 trials. Avoiding cannibalism of males in a species that appears to require multiple males for each polyandrous female, would allow males to remain in the population longer.

While the *M. inclemens* female's preference for alternative male morphs over multiple matings is supported, a larger study would strengthen the hypothesis. Future studies of field behavior and comparison of reproductive strategies with other members of the genus *Maevia* and other Salticidae should further enhance our understanding of the evolution of this reproductive system, specifically, why do *Maevia* females have brief copulations and prefer novel males?

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Appendix 1. Data

Time (duration) measured in seconds.

treatment	trial	male morph	duration of mount including palp insertion	duration of palp insertion	time to mount after female orientation to male	number of male pounces preceding mount
FM	1	gray	5	1	36	0
FM	2	tufted	10	2	10	1
FM	3	tufted	19	11	8	2
FM	4	tufted	16	5	209	3
FM	5	tufted	10	2	90	0
FM	6	gray	126	50	27	0
FM	7	gray	44	42	45	0
FM	8	tufted	11	1	65	0
FM	9	gray	10	2	64	0
FM	10	gray	26	3	40	2
FM	11	gray	32	12	26	0
FM	12	tufted	21	14	209	2
FM	13	gray	17	2	12	3
FM	14	gray	13	8	27	0
FM	15	tufted	16	7	70	1
FM	16	gray	2	2	180	0
FM	17	tufted	25	4	15	0
FM	18	gray	9	3	90	3
FM	19	tufted	48	36	510	6
FM	20	gray	4	0	90	0
FM	21	tufted	3	2	40	0
FM	22	tufted	33	3	4	1
FM	23	gray	10	3	38	0
FM	24	tufted	3	0	252	0
FM	25	gray	7	6	11	0
SI	1	gray	0	0	NA	NA
SI	2	tufted	0	0	NA	NA
SI	3	gray	0	0	NA	NA
SI	4	gray	44	15	25	1
SI	5	tufted	10	2	510	6
SI	6	tufted	45	11	110	3
SI	7	tufted	6	1	320	4
SI	8	gray	12	10	13	0
SI	9	tufted	3	1	390	0
SI	10	tufted	3	2	180	1
NS	1	tufted	0	0	NA	NA
NS	2	tufted	0	0	NA	NA
NS	3	tufted	8	3	20	1
NS	4	tufted	5	1	117	3
NS	5	gray	7	2	501	6
NS	6	gray	9	1	260	8
NS	7	gray	9	2	452	7
NS	8	gray	12	4	291	9
NS	9	tufted	6	3	92	0
NS	10	tufted	27	18	64	1

treatment	trial	male morph	duration of mount including palp insertion	duration of palp insertion	time to mount after female orientation to male	number of male pounces preceding mount
NS	11	tufted	50	21	31	2
NS	12	tufted	31	12	75	2
NS	13	tufted	6	2	26	0
NS	14	tufted	11	2	66	1
NS	15	tufted	6	1	51	2
NS	16	tufted	7	2	48	4
NS	17	gray	3	1	116	2
NS	18	gray	9	2	11	2
NS	19	gray	20	12	34	3
NS	20	gray	6	2	54	2
NS	21	gray	12	7	68	0
NS	22	gray	3	1	145	2
NS	23	gray	6	1	76	0
NS	24	gray	20	12	126	0
NS	25	tufted	40	28	45	1
NS	26	gray	18	4	90	0
NS	27	tufted	32	15	27	3
NS	28	gray	0	0	NA	NA
NS	29	gray	0	0	NA	NA
NS	30	gray	3	0	131	1
NS	31	gray	5	2	135	1
NS	32	gray	12	4	38	0
NS	33	gray	0	0	NA	NA
NA	1	gray	0	0	NA	NA
NA	2	tufted	9	3	360	0
NA	3	gray	48	16	90	5
NA	4	gray	35	25	7	3
NA	5	tufted	40	8	153	4
NA	6	gray	10	7	6	0
NA	7	gray	12	5	31	0
NA	8	gray	33	27	21	1
NA	9	tufted	60	46	29	0
NA	10	gray	16	13	22	3
NA	11	tufted	87	26	15	1
NA	12	gray	10	4	89	2
NA	13	tufted	17	2	30	3
NA	14	gray	44	20	15	4
NA	15	tufted	24	9	16	1
NA	16	tufted	41	7	60	1
NA	17	gray	47	27	13	0
NA	18	gray	17	4	23	0
NA	19	tufted	18	3	5	2
NA	20	gray	2	1	78	0
NA	21	tufted	15	9	50	0
NA	22	gray	50	11	132	5