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TEMPORAL MATING PATTERNS OF THE FALL ARMYWORM *Spodoptera frugiperda* (LEPIDOPTERA: NOCTUIDAE) POPULATIONS IN INDONESIA: IMPLICATIONS FOR STRAIN INFERENCE AND PHEROMONE COLLECTION

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ABSTRACT

The fall armyworm (*Spodoptera frugiperda*), an invasive noctuid pest first reported in Indonesia in 2019, poses a major threat to maize production. In its native range, two host-associated strains, corn and rice, are recognized, differing in both pheromone blend composition and nocturnal mating time. Understanding the mating behavior of local populations is critical for optimizing pheromone extraction and developing locally adapted lures. This study aimed to describe the nocturnal mating timing of Indonesian populations of *S. frugiperda*, assess its relevance as a behavioral indicator of strain identity, and identify an optimal scotophase window for pheromone-gland extraction. The results showed that the nocturnal mating time of *S. frugiperda* in Indonesia is concentrated in the early scotophase (≈3–6 h) and is more consistent across populations than mating frequency or success. Using circular statistics and generalized linear models under common-garden conditions, we quantified clear phase differences across nine populations while noting limited power to resolve concentration (κ) in low-activity groups. These findings provide a practical framework to standardize pheromone-gland extraction within a 3–6 h window after scotophase onset, which can reduce chemical variance and accelerate lure design. We propose this behavioral indicator as a decision-support tool, indicative rather than diagnostic, and recommend that it be combined with genotyping and pheromone profiling for reliable strain assignment. Local validation remains essential, especially in populations with low activity concentration. Future research should combine mating-time assessments with pheromone composition analysis and field studies using lures calibrated to the early-night schedule, thereby improving the practicality of pheromone-based monitoring and control in Indonesia.

Keywords: Allochronic; integrated pest management; prezygotic isolation; scotophase behavior; sex pheromone

ABSTRAK

Ulat ratus tentera (*Spodoptera frugiperda*), serangga perosak Noctuidae invasif yang mula dilaporkan di Indonesia pada 2019 merupakan ancaman utama terhadap pengeluaran jagung. Dua strain yang berasosiasi dengan perumah iaitu jagung dan padi, dikenal pasti yang berbeza dari segi komposisi campuran feromon serta masa mengawan pada waktu malam. Memahami tingkah laku mengawan populasi tempatan adalah penting untuk mengoptimumkan pengekstrakan feromon serta membangunkan umpan yang disesuaikan dengan keadaan tempatan. Kajian ini bertujuan untuk menghuraikan masa mengawan nokturnal populasi *S. frugiperda* di Indonesia, menilai kerelevanannya sebagai penunjuk tingkah laku bagi identiti strain, serta mengenal pasti tempoh skotofasa yang optimum untuk pengekstrakan kelenjar feromon. Hasil kajian menunjukkan bahawa masa mengawan nokturnal *S. frugiperda* di Indonesia tertumpu pada awal skotofasa (≈3–6 jam) dan lebih konsisten antara populasi berbanding kekerapan atau kejayaan mengawan. Dengan menggunakan statistik bulatan dan model linear tergeneralisasi di bawah keadaan *common-garden*, kami mengkuantifikasi perbezaan fasa yang jelas merentasi sembilan populasi, sambil mendapati kuasa analisis yang terhad untuk menentukan kepekatan (κ) dalam kumpulan dengan aktiviti rendah. Dapatan ini menyediakan kerangka praktikal untuk menyeragamkan pengekstrakan kelenjar feromon dalam tempoh 3–6 jam selepas permulaan skotofasa, yang berpotensi mengurangkan varians kimia serta mempercepat pembangunan umpan. Kami mencadangkan penunjuk tingkah laku ini sebagai sokongan keputusan yang bersifat indikatif, bukannya diagnostik dan mengesyorkan agar ia digabungkan dengan genotip serta pemprofilan feromon bagi penentuan strain yang lebih boleh dipercayai. Pengesahan setempat masih penting, terutamanya dalam populasi yang mempunyai kepekatan aktiviti rendah. Kajian masa hadapan disarankan untuk menggabungkan penilaian masa mengawan dengan analisis komposisi feromon serta kajian lapangan menggunakan umpan yang dikalibrasi kepada jadual awal malam, sekali gus meningkatkan kepraktisan pemantauan dan kawalan berasaskan feromon di Indonesia.

Kata kunci: Alokroni; pengurusan perosak bersepadu; pengasingan prazigotik; tingkah laku skotofasa; feromon seks

INTRODUCTION

The fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), has emerged as a major invasive pest threatening maize production in tropical Asia, including Indonesia (Kenis et al. 2023; Sartiami et al. 2020). FAW is native to the Americas and has a broad host range of more than 350 plant species, with a preference for maize (Cruz-Esteban et al. 2020; Cruz-Díaz et al. 2022; Luqmana et al. 2024). Since 2016, it has expanded rapidly across Africa and Asia, presenting severe risks to food security (Goergen et al. 2016; Tay et al. 2023). The first detection of FAW in Indonesia was reported in 2019 (Sartiami et al. 2020). It caused significant damage and rapidly disseminated to regions where maize is extensively cultivated (Maharani et al. 2021; Subagyo et al. 2025). These impacts highlight the necessity for reliable, field-relevant approaches to support monitoring and management.

The fall armyworm comprises two morphologically indistinguishable but genetically and ecologically distinct strains: the corn strain (C-strain) and the rice strain (R-strain) (Nagoshi & Meagher 2022; Schöfl et al. 2009). They differ in host-plant preference, mating behavior, pheromone composition, and insecticide resistance (Boaventura et al. 2020; Schöfl et al. 2009). The most prevalent methods for strain identification are nuclear (Tpi) and mitochondrial (COI, COII) markers (Nagoshi 2010). However, discordance between mitochondrial and nuclear markers has been widely reported, which can result in ambiguous strain assignment (Dharmayanthi et al. 2022; Fahmi et al. 2023). In Indonesia, there are cases of incongruence in which individuals possess the R-strain COI haplotype alongside the C-strain Tpi genotype (Fahmi et al. 2023). This situation complicates strain-specific management and pheromone-based monitoring (Groot et al. 2008; Kenis et al. 2023). Consequently, dependence solely on genetics may be inadequate for operational judgments, especially in contexts where marker incongruence is common.

Pheromone-based approaches are increasingly used in FAW monitoring and management, and mating-time differences between strains may provide complementary behavioral information in addition to molecular markers (Batista-Pereira et al. 2006; Kenis et al. 2023). The C-strain usually mates earlier, while the R-strain is active later at night (Schöfl et al. 2009; Schöfl et al. 2011). These temporal differences are associated with prezygotic isolation and are also relevant to pheromone collection because gland activity peaks during calling (Unbehend et al. 2013). Genomic and behavioral research demonstrates that FAW strains are genetically distinct yet capable of hybridization, with reproductive isolation partially maintained through differences in nocturnal activity patterns (Schöfl et al. 2011; Tessnow et al. 2022; Unbehend et al. 2013). Comprehending strain-associated behavior is crucial for the creation of pheromone-based tools, such as monitoring, mass trapping, and mating disruption (Batista-Pereira et al. 2006; Kenis et al. 2023).

Indonesian FAW populations have not been systematically characterized for nocturnal mating schedules, creating a local evidence gap that this study seeks to address. This gap is important because standardized knowledge of mating time may help improve the consistency of pheromone-related sampling and support subsequent chemical analyses. While strain-associated timing differences are well documented, local schedules may vary under different environmental conditions; thus, laboratory timing should be interpreted as decision support rather than a diagnostic tool. Accordingly, a locally validated early-night sampling window may help reduce variation in pheromone-gland samples and improve the efficiency of downstream analyses.

Here, we address this gap by characterizing nocturnal mating timing and associated reproductive traits in nine Indonesian FAW populations maintained under standard conditions. Specifically, this study aims to (i) characterize the distribution of copulation timing during the dark phase (scotophase), (ii) provide behavioral evidence relevant to strain identity, and (iii) identify a pragmatic scotophase window for pheromone gland extraction. We hypothesize that Indonesian FAW populations exhibit mating activity concentrated in the early scotophase and that this temporal pattern can provide practical guidance for behavioral inference and pheromone sampling.

MATERIALS AND METHODS

Experimental Populations and Rearing

Fall armyworm larvae were collected from nine maize fields in East Java (December 2023) and Lampung (July 2024) (locations in Figure 1; details in Table 1) and reared as separate colonies at the Cikeumeuh insect rearing laboratory (BBPSI Biogen, Bogor) under 29±1°C, 65±5% RH, and a 12:12 h L:D photoperiod. Rearing followed Sianturi et al. (2022): Early instars were kept in plastic containers (30×20×7 cm) with baby corn; late instars were transferred to containers with sawdust for pupation. Pupae were placed in jars (15 cm diameter) lined with filter paper, covered with gauze, and provided with a 10% honey solution. To ensure virginity, pupae were sexed and held individually until emergence. Behavioral observations were performed with F2–F3 generations.

Table 1. Sampling locations and sample sizes of *Spodoptera frugiperda* populations in Lampung and East Java, Indonesia

Province	**Population Code**	**Location Coordinates**[a]	**Pairs Set Up (n)**	**Pairs Mating (n)**
Lampung	LS1 (South Lampung 1)	5.269861° S, 105.398000° E	25	10
	LS2 (South Lampung 2)	5.236944° S, 105.287583° E	20	11
	LG (Central Lampung)	5.047972° S, 105.285194° E	19	13
	LT (East Lampung)	5.006639° S, 105.489833° E	12	7
East Java	JB (Jember)	8.342139° S, 113.613000° E	10	5
	ML (Malang)	8.120973° S, 112.633583° E	30	15
	KD (Kediri)	7.823667° S, 112.063722° E	20	13
	BW (Banyuwangi)	8.287805° S, 113.980528° E	16	8
	SP (Sumenep)	6.897222° S, 113.741944° E	12	5

[a] Coordinates in decimal degrees (WGS84)

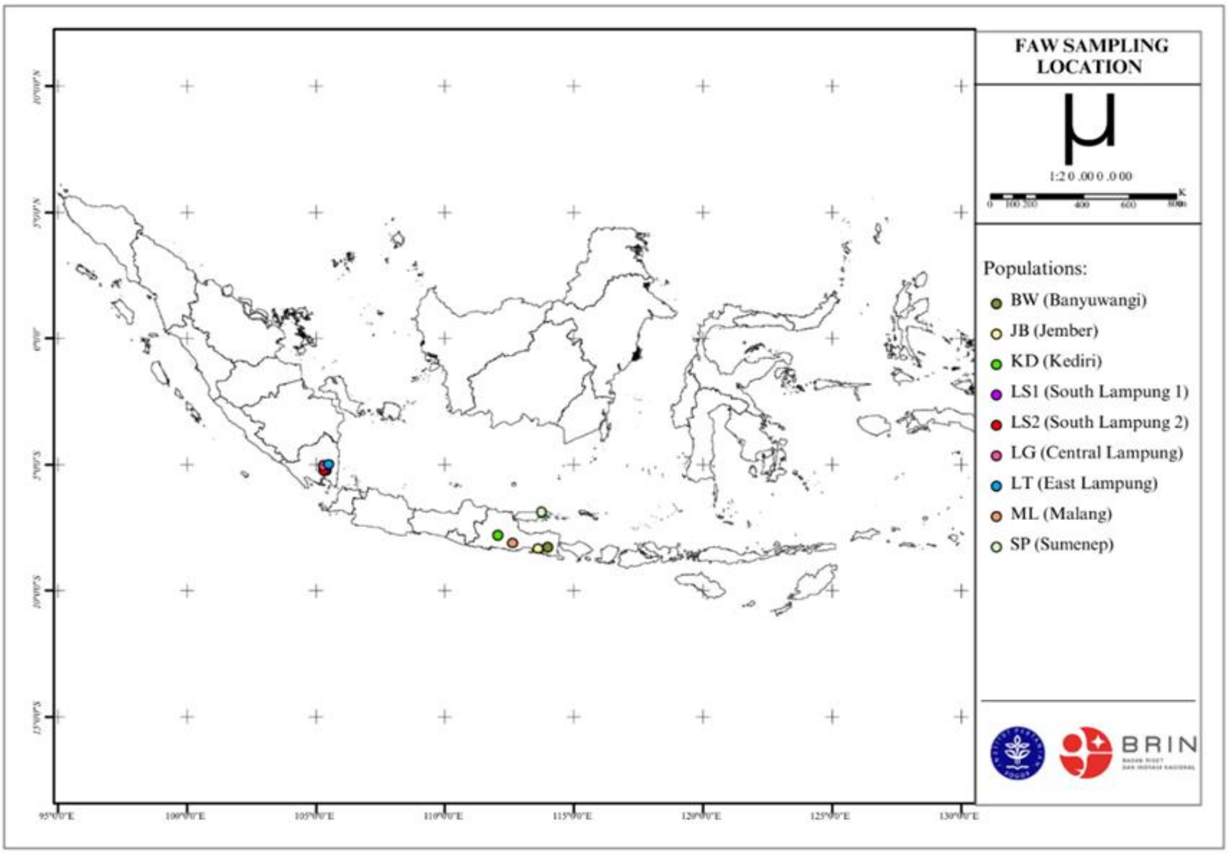

Figure 1. Sampling sites of *Spodoptera frugiperda* populations in Lampung and East Java, Indonesia. Colored dots indicate the nine populations used for copulation behavior assays (see Table 1 for coordinates)

Photoperiod and Environmental Conditions

Observations were conducted in a walk-in chamber at 28.7±1.0°C and 76.3±5% RH under dim red light < 1 lx (peak 620–660 nm), verified with a lux meter. For daytime scoring, the photoperiod was shifted so that the dark phase (scotophase) ran from 06:00 to 18:00 local time (UTC+07:00); lights-off defined scotophase 0 h, and all timing variables were expressed as hours into scotophase (h).

Pairing and Allocation (Operational Randomization)

On each observation night, mating pairs were established using virgin adults 1–3 d post-emergence. Females (emerging earlier) were staged first (typically 1–2 d post-emergence); males were introduced 1–2 d later so both remained virgins within a 1–3 d window. Within each population and night, males were randomly assigned to females (simple draw without replacement); each individual was used once.

Observation of Nocturnal Reproductive Behaviors

One male and one female were placed in clear 16 oz cups (≈ 9 × 15 cm) with maize leaves and 10% honey solution. Pairs were checked every 30 min throughout the 12 h scotophase; each examination lasted ≈ 3–5 s under red light. A 30 min interval was chosen after pilot comparisons (15 vs 30 min), which showed no difference in circular mean times. Monitoring continued nightly until a member died or activity ceased. All observations were performed by a single trained observer using a standardized protocol.

Recorded Variables and Data Visualization

For each pair we recorded copulation success (mated/unmated), copulation frequency (total copulations, including zeros), precopulatory period (days from emergence to first copulation), copulation duration (h), and male/female age at mating (days) (Supplementary Table S1). To preserve statistical independence, timing analyses were based on the first-event copulation onset per pair (hours into scotophase; Supplementary Table S2). Onset times were binned at 0.5 h (0–12 h) for histograms; ECDFs of first-event onsets were also plotted. No pheromone extraction or chemical analyses were performed in this study; any 3–6 h reference is behavioral and intended as operational guidance pending chemical validation. Pairwise contrasts for frequency models are provided in Supplementary Table S3. Population-level frequency summaries (n, mean frequency, proportion of zeros) are in Supplementary Table S4; model diagnostics (AIC, residual deviance, dispersion, DHARMa) are reported in the Table 3 footnote. Population-specific Monte-Carlo sensitivity (power) for Rayleigh's test is provided in Supplementary Table S5.

Statistical Analysis

Analyses were conducted in R 4.4.3 (RStudio) using stats, MASS (glm.nb), DHARMa, car (type-II LR tests), emmeans, rstatix, effectsize, broom, and circular/CircStats. $\alpha = 0.05$; Holm adjustments for multiple comparisons.

Count and Binary Outcomes

Frequency (including zeros) used GLMs with a log link; overdispersion was assessed via Pearson residual deviance/df and DHARMa; Poisson was used when acceptable, otherwise negative binomial (MASS:glm.nb). Success (mated vs. unmated) used a binomial GLM (logit). Population was the main fixed predictor (reference = LS1); male/female age and the precopulatory period were included as covariates when applicable. We report IRR/OR (95% CI), type II LR omnibus tests, and Holm-adjusted pairwise contrasts from emmeans. Population contrasts on frequency: the main text reports LS1-referenced contrasts (NB-GLM; profile-likelihood 95% CI; exact p); Supplementary S3 provides Tukey all-pairs (numerator/denominator), so significance may differ.

Continuous Outcomes

Normality (Shapiro–Wilk; Q–Q) and homogeneity (Levene/Brown–Forsythe) guided ANOVA (η^2, 95% CI) or Kruskal–Wallis (ε^2); significant omnibus tests had Holm-adjusted post hoc comparisons.

Circular Timing Analyses

For each pair, first onset (h) was converted to radians ($\theta = h/12 \times 2\pi$). For each population we computed μ (back-transformed to h), r, and 95% CI; Rayleigh's test assessed non-uniformity. Watson–Williams was used when κ homogeneity held; otherwise, Mardia–Watson–Wheeler. Population-specific metrics and the κ diagnostic are presented in Table 5.

Fixed-Effects Rationale and Reproducibility

Population was treated as fixed (named localities of a priori interest; unbalanced replication complicates variance components), so inference is conditional on sampled populations. Random nightly pairing reflects real-time availability. All scripts (with package versions) are archived; tests were two-sided.

RESULTS AND DISCUSSION

Timing of First Mating (Overview and Omnibus Tests)

First-event copulation onsets were concentrated in the early scotophase across nine populations (n = 164 pairs; 87 mated). Circular mean phases ranged from 3.1 to 5.9 h (r = 0.28–0.85), and 8 of 9 Rayleigh tests were significant ($p<0.05$). Among-population phase differences were significant (Mardia–Watson–Wheeler W = 46.52, df = 16, $p<0.001$; Watson–Williams F = 7.03, df1 = 8, df2 = 78, $p<0.001$ when κ-homogeneity held). Copulation success did not vary among populations, and frequency differences were modest, higher in LS2 (IRR 2.60 [95% CI 1.26, 5.56]) and LG (IRR 3.14 [95% CI 1.55, 6.66]) relative to LS1, supporting timing as the clearest among-population signal under common-garden conditions. The population effect on copulation frequency was significant (type-II LR χ^2 = 15.72, df = 8, p=0.047), whereas copulation success showed no population effect (χ^2 = 5.74, df = 8, p=0.677) (Figure 2–4; Tables 3 & 5).

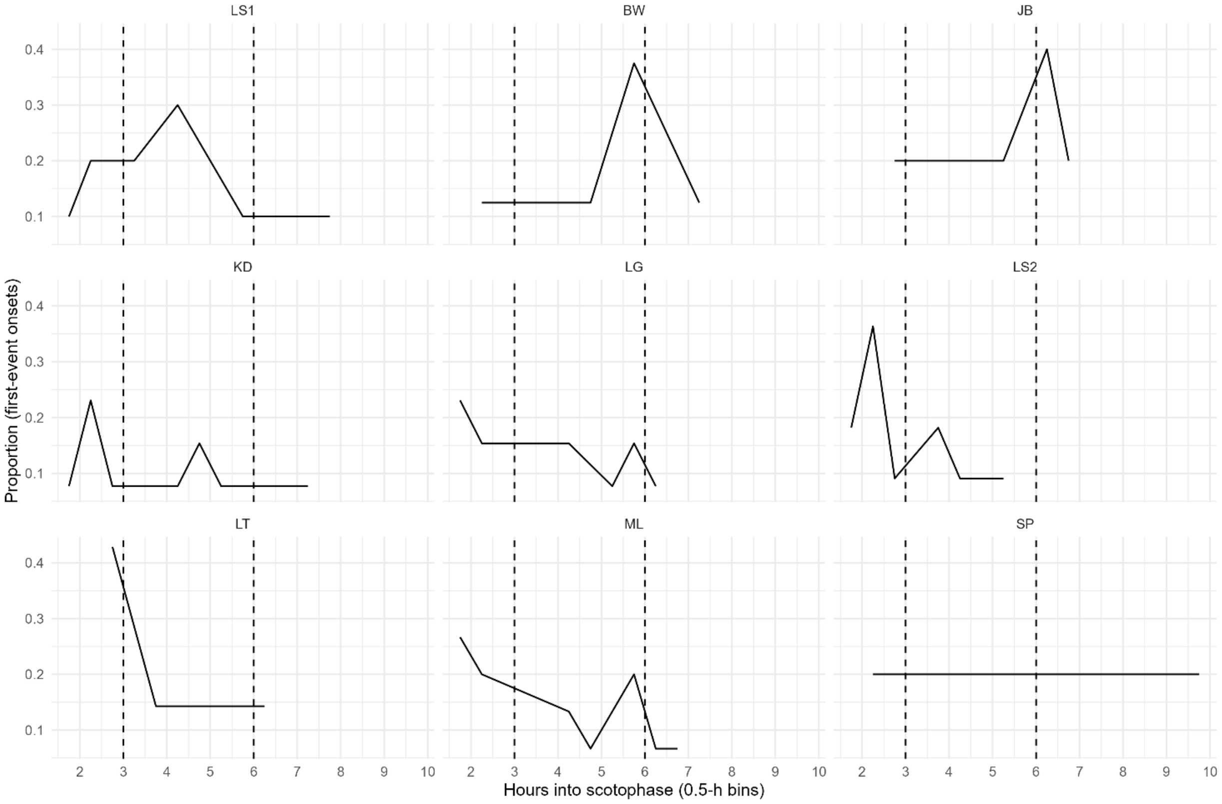

Figure 2. Temporal distribution of first-event copulation onsets (0.5-h bins) during the 12 h scotophase across nine populations (n = 87 mated pairs)

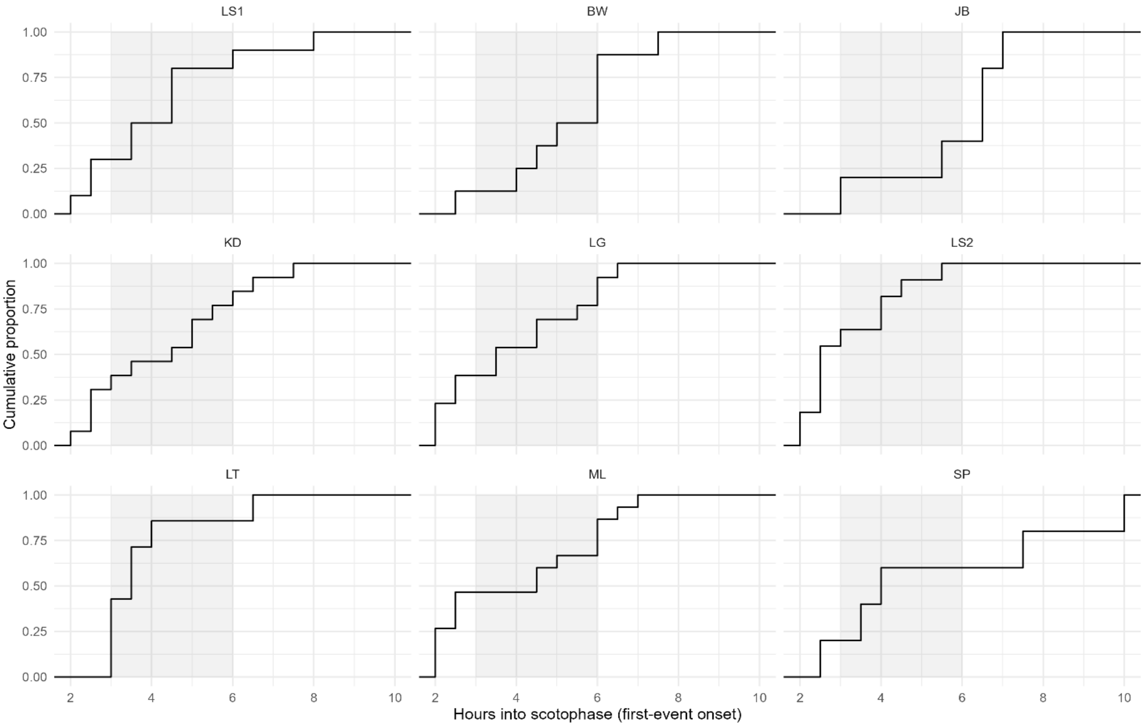

Figure 3. Empirical cumulative distribution (ECDF) of first-event copulation onsets across nine populations (n = 87 mated pairs), plotted as h into scotophase (x-axis) and cumulative proportion (y-axis). See Table 5 for circular statistics and among-population tests

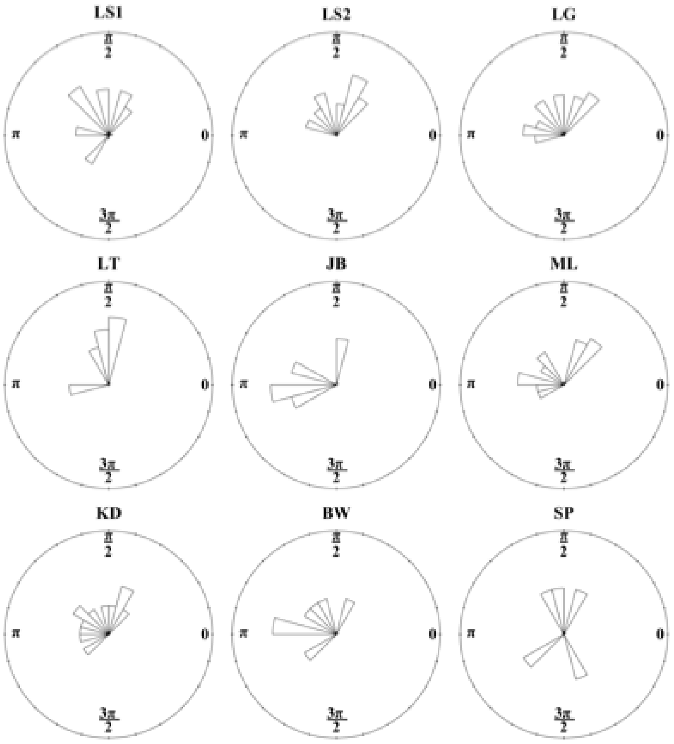

Figure 4. Rose diagrams of first-event copulation onsets for nine populations. Angles denote h since lights-off (wrapped to 2π); radial length scales with frequency. Most activity was concentrated within the early scotophase, with broader/later profiles in JB and BW and a dispersed pattern in SP

Copulation Behavior Parameters

Table 2 summarizes means±SD with 95% CI for four pair-level traits among mated pairs: precopulatory period, copulation duration, and male/female age at mating. Copulation duration was broadly conserved across populations (≈ 1.2–2.1 h, overlapping CI), and mating typically occurred 1–3 d post-emergence (grand means ≈ 1.9 d for males and 2.4 d for females) (Table 2). The clearest among-population structure was in the precopulatory period: LG showed the shortest mean interval (1.30±1.00 d), whereas several populations (e.g., KD, LS1) averaged >2 d (Table 2). These descriptive contrasts motivated the formal omnibus tests (Table 4).

Table 2. Pair-level behavioral traits among mated pairs: Means±SD and 95% CI for precopulatory period (d), copulation duration (h), and male and female age at mating (d) by population

Population	**n (pairs)**[a]	**Parameter**							
		Precopulatory Period (d)		**Copulation Duration (h)**		**Male Mating Age (d)**		**Female Mating Age (d)**	
		Mean±SD	**95% CI**	**Mean±SD**	**95% CI**	**Mean±SD**	**95% CI**	**Mean±SD**	**95% CI**
LS1	10	2.29±0.61	1.85–2.73	2.05±1.45	1.01–3.09	1.90±0.57	1.49–2.31	2.30±0.95	1.62–2.98
LS2	11	1.59±1.12	0.84–2.34	1.32±0.49	1.00–1.65	1.64±1.29	0.77–2.50	1.91±1.45	0.94–2.88
LG	13	1.30±1.00	0.70–1.91	1.44±0.68	1.02–1.85	1.23±0.73	0.79–1.67	2.00±1.35	1.18–2.82
LT	7	1.35±0.92	0.51–2.20	1.29±0.72	0.62–1.95	1.86±1.07	0.87–2.85	2.14±1.07	1.15–3.13
JB	5	2.15±1.06	0.83–3.46	1.20±0.76	0.26–2.14	2.00±1.00	0.76–3.24	2.20±1.10	0.84–3.56
ML	15	2.02±0.96	1.49–2.55	1.89±0.90	1.39–2.39	1.73±1.28	1.02–2.44	2.67±1.23	1.98–3.35
KD	13	2.78±1.24	2.03–3.53	1.29±0.48	1.00–1.58	2.62±1.45	1.74–3.49	3.54±1.61	2.56–4.51
BW	8	1.49±0.54	1.04–1.95	1.19±0.75	0.56–1.82	1.50±0.53	1.05–1.95	2.00±1.07	1.11–2.89
SP	5	1.54±1.21	0.04–3.04	1.25±0.75	0.32–2.18	2.20±1.10	0.84–3.56	2.60±0.89	1.49–3.71

[a] n = number of pairs that mated at least once in the population; [b] Means and 95% CI are computed per population for each parameter

Table 3. GLM results: Event frequency (negative-binomial) and mating success (logistic)

Parameter[a]	**Term**	**Effect Size**	**95% CI**	***P*-Value**	**Interpretation**
Copulation frequency[b]	Population LS1	IRR = 1.00	–	-	Reference (baseline)
	Population LS2	2.60	1.26–5.56	0.011	Significant
	Population LG	3.14	1.55–6.66	0.002	Significant
	Population LT	2.08	0.88–4.95	0.093	Suggestive, not significant
	Population JB	1.54	0.56–4.00	0.383	Not significant
	Population ML	1.35	0.64–2.91	0.437	Not significant
	Population KD	1.73	0.80–3.85	0.169	Not significant
	Population BW	1.44	0.61–3.41	0.402	Not significant
	Population SP	1.44	0.55–3.63	0.440	Not significant
Copulation success[c]	Population LS1	OR = 1.00	-	-	Reference (baseline)

Parameter[a]	**Term**	**Effect Size**	**95% CI**	***P*-Value**	**Interpretation**
	Population LS2	1.83	0.56–6.19	0.318	Not significant
	Population LG	3.25	0.96–12.06	0.066	Suggestive, not significant
	Population LT	2.10	0.52–8.97	0.299	Not significant
	Population JB	1.50	0.34–6.77	0.590	Not significant
	Population ML	1.50	0.52–4.47	0.459	Not significant
	Population KD	2.79	0.84–9.85	0.099	Suggestive, not significant
	Population BW	1.50	0.42–5.43	0.530	Not significant
	Population SP	1.07	0.25–4.35	0.923	Not significant

[a]IRR/OR relative to LS1; CI are profile-likelihood; omnibus p-values are from LR tests; the frequency model includes zeros; [b] Omnibus LR test (Population): $\chi^2 = 15.72$, df = 8, $p = 0.047$; [c] Omnibus LR test (Population): $\chi^2 = 5.74$, df = 8, $p = 0.677$. Model fit (frequency): AIC = 261.4; residual deviance = 34.6 on 78 df; Pearson $\chi^2/\mathrm{df} \approx 1$; DHARMa checks showed no QQ, overdispersion, or zero-inflation issues

Table 4. Omnibus comparisons of pair-level traits among populations (test type specified per trait). Reported test statistics (df and *p*) and indicated post-hoc method if used

Parameter	**Test**	**Test Statistic (Df)**	**Effect Size**	**95% CI**	***P*-Value**
Precopulatory period	ANOVA	F = 2.786 (8, 78)	$\eta^2 = 0.222$	0.038–1.000	0.009
Copulation duration	ANOVA	F = 1.032 (8,78)	$\eta^2 = 0.096$	0.000–1.000	0.420
Male mating age	Kruskal–Wallis	H = 13.620 (8)	$\varepsilon^2 = 0.072$	-	0.092
Female mating age	ANOVA	F = 1.915 (8,78)	$\eta^2 = 0.164$	0.000–1.000	0.069

Table 5. Circular statistics by population for first-event onsets: circular mean phase (μ, h since lights-off), concentration (r), 95% CI for μ, and Rayleigh *p*-values; summary of among-population circular tests

Population	**n (pairs)**	**Mean phase (h)**[a]	**Mean resultant length (r)**[b]	**Rayleigh Z**[c]	**Rayleigh *p***[c]	**Kappa (κ; dispersion)**
LS1	10	3.92	0.675	4.56	0.0072	1.861
LS2	11	3.13	0.847	7.89	0.0000	3.585
LG	13	3.87	0.674	5.91	0.0015	1.857
LT	7	3.66	0.841	4.95	0.0031	3.464

Population	**n (pairs)**	**Mean phase (h)**[a]	**Mean resultant length (r)**[b]	**Rayleigh Z**[c]	**Rayleigh *p***[c]	**Kappa (κ; dispersion)**
JB	5	5.88	0.754	2.85	0.0499	2.399
ML	15	4.00	0.579	5.02	0.0049	1.424
KD	13	4.23	0.648	5.45	0.0027	1.721
BW	8	5.24	0.749	4.49	0.0069	2.356
SP	5	4.00	0.283	0.40	0.6924	0.590

[a] Mean phase is h into the scotophase; [b] r = mean resultant length (0–1); κ = concentration parameter (larger = more clustered); [c] Rayleigh's test evaluates departure from uniformity for each population.

Generalized Linear Models

A binomial GLM for copulation success detected no population effect (LR omnibus *p*=0.677); all pairwise odds ratios were non-significant after Holm adjustment (LG *p*=0.066; KD *p*=0.099 are suggestive, not significant). For event frequency, the negative-binomial GLM indicated modest among-population variation (AIC = 261.4; residual deviance = 34.6 on 78 df; Table 3). Relative to LS1, LS2 (IRR = 2.60, *p*=0.011) and LG (IRR = 3.14, *p*=0.002) showed elevated rates, whereas other contrasts were non-significant (*p>0.30*; Table 3). These patterns support timing as the clearer among-population signal under common-garden conditions. Full LS1-referenced contrasts are provided in Supplementary Table S3 (Tukey all-pairs in S3b), model-fit diagnostics appear in the Table 3 footnote, and descriptive frequency summaries are in Supplementary Table S4.

Temporal Patterns and Circular Analysis

Empirical cumulative distribution functions (ECDFs) show a rapid rise in first-mating onsets during the first half of the night, with most events accumulating within the early-night band (Figure 3). Rose diagrams visualize population-level shape differences: several populations display narrow early-night peaks, whereas others (e.g., JB, BW) have broader or later-skewed profiles, and SP shows a dispersed, low-concentration pattern (Figure 4). These graphical patterns align with the summary statistics reported above and support an operational early-night window under common-garden conditions.

For among-population inference on circular means, an initial Watson–Williams screen indicated differences in phase. However, concentration parameters (κ) varied among populations (see Table 5), violating the homogeneity assumption of the Watson–Williams test. We therefore relied on the Mardia–Watson–Wheeler test, which does not assume equal κ and likewise indicated significant among-population differences (Figure 2; Table 5). Taken together, the ECDFs, rose diagrams, and assumption-appropriate tests converge on the same conclusion: timing differences exist across populations, but most activity is concentrated in the early scotophase.

Rayleigh's Test (Per Population)

To aid interpretation, we estimated the population-specific sensitivity of Rayleigh's test via Monte-Carlo simulation (5,000 replicates; $\alpha = 0.05$) using each population's observed sample size (*n*) and concentration (*r*). Sensitivity was high for most populations (LS2 ≈ 1.000; LT ≈ 0.983; LG ≈ 0.964; KD ≈ 0.941; BW ≈ 0.914; ML ≈ 0.899; LS1 ≈ 0.893), moderate for JB (≈ 0.700), and low for SP (≈ 0.109); 95% Monte-Carlo CI are provided in Supplementary Table S5. These estimates align with the empirical Rayleigh outcomes (8/9 significant; SP non-significant) and support cautious interpretation where *n* is small and/or *r* is low. Across analyses, FAW mates (first-event onset and circular phase) exhibited a clearer and more consistent among-population structure compared to their mating frequency under shared laboratory conditions, while basic performance traits (copulation duration and mating ages) remained largely conserved.

Our primary result is that timing, defined as when FAW mates within the scotophase, provides the clearest among-population signal under shared laboratory conditions. Copulation success did not differ among populations, whereas copulation frequency showed a modest population effect driven by significantly higher rates in LS2 and LG relative to LS1. These rate differences did not translate into shifts in nightly phase, which remained clustered in the early scotophase across populations.

The ~50% mating success observed in this study is consistent with previous laboratory studies reporting that not all pairs copulate and that remating is low to moderate (Lima & McNeil 2009; Liu et al. 2024; Ramya et al. 2024; Simmons & Rogers 1994). The elevated frequency in LS2 and LG was consistent with among-population differences in mating propensity rather than changes in courtship structure or phase. In contrast, independence-aware timing metrics (first-event onset and circular mean phase) revealed strong early-night clustering, underscoring that when FAW mates, it provides more population signal than how often it mates under common-garden conditions (Hänniger et al. 2017; Schöfl et al. 2009; Schöfl et al. 2011; van Doorn et al. 2024).

Our findings align with the observed trend that the corn strain exhibits higher nocturnal activity compared to the rice strain (Nagoshi & Meagher 2022; Schöfl et al. 2009; Schöfl et al. 2011), with the bulk of first onsets and circular means occurring 3–6 h into the scotophase (Table 5). This allochrony has been identified as a prezygotic isolating mechanism that persists despite gene flow (Hänniger et al. 2017; Tessnow et al. 2022) and corresponds with the pheromonal divergence observed between strains (Groot et al. 2008; Unbehend et al. 2013). Regionally, shifts in peak timing are known (for example, Africa vs. the Americas), but they generally remain within an early-night strategy (Haenniger et al. 2020), supporting the need for local behavioral calibration before chemical or field deployment.

In this study, timing is interpreted as supportive evidence for sampling design rather than as a stand-alone diagnostic criterion for strain identity. It helps standardize the sampling window for pheromone-gland extraction and informs lure testing schedules, but it is not a behavioral diagnostic of strain identity. Accordingly, timing should be paired with chemical profiling and genetics when strain assignment is important.

The early-night concentration of mating provides actionable guidance to initiate pheromone-gland sampling 3–6 h after local sunset as a working hypothesis. This study did not prepare or validate extraction protocols, GC–MS workflows, or field lure trials. Therefore, we do not prescribe procedural details. As a pragmatic next step, we recommend a short local pilot to confirm the calling and mating peak, followed by calibration of conditions, then chemical and field validation under institutional SOPs.

Operationally, the convergence of onsets and phases within 3–6 h had immediate value. Female calling and pheromone release overlap with mating activity (Batista-Pereira et al. 2006; Cruz-Díaz et al. 2022), and studies in other regions likewise observed strong emissions around the fourth to sixth h (Akter et al. 2025) and recovered strain-specific blends when sampling approximately 3h and 6h (Cañas-Hoyos et al. 2017). We recommend standardizing pheromone-gland extraction at 3–6 h into scotophase for GC–MS characterization and subsequent field validation, in conjunction with the development of locally tuned lures (Kenis et al. 2023).

Keeping zeros in the frequency model was appropriate and allowed us to detect modest rate differences. Reporting type-II LR omnibus tests and response-scale CIs (IRR/OR) increases transparency, and simulation-based residual diagnostics supported GLM adequacy. Although zero-inflated and mixed-effects formulations were attempted as sensitivity analyses, they did not converge under the modest and unbalanced design. We therefore retained GLMs as the most stable option while acknowledging residual zero-inflation. Equally important, our independence-preserving approach (one first-event datum per pair for timing) minimized pseudoreplication, consistent with recommendations for nocturnal behavior in FAW (Liu et al.

2024; Schöfl et al. 2009). Future work should explore hierarchical models to partition among population variances.

These behavioral results are consistent with genetic surveys reporting corn-strain dominance in Indonesia, but we treat them as complementary indicators rather than diagnostic evidence, since behavioral timing alone cannot confirm strain identity (Dharmayanthi et al. 2022; Fahmi et al. 2023; Tay et al. 2023). They help reconcile marker incongruence by offering a quick, field-portable indicator that complements genotyping rather than replaces it (Nagoshi 2010; Nagoshi & Meagher 2022; Tessnow et al. 2022). Timing is an operational marker rather than a diagnostic of strain identity; field validation with pheromone chemistry and trap performance remains necessary. Taken together, these findings suggest that behavioral timing may serve as a useful operational indicator for sampling and monitoring, while remaining insufficient as a sole basis for strain assignment.

Our Indonesia-wide common-garden comparison isolates among-population differences in mating phase from local weather and photoperiod. Relative to previous multi-region reports of FAW allochrony, we delineate a practical early-night window and show that phase provides a clearer among-population structure than mating frequency or success. Timing is an informative operational indicator, not a diagnostic of strain or origin; any operational use should be paired with genotyping and pheromone chemistry. Power to detect differences in concentration (κ) was limited in low-activity populations, so estimates for those groups should be interpreted cautiously. For applications, field monitoring and lure deployment should prioritize 3–6 h after scotophase onset, with local validation where concentration is low.

We met our objectives by (i) demonstrating early-night mating concentrated in 3–6 h into scotophase across Indonesian populations; (ii) showing that onset/phase, rather than success (with only a modest effect on frequency) carried the clearest population signal under shared conditions; and (iii) proposing a practical 3–6 h scotophase window to guide pheromone-gland sampling and to accelerate GC–MS characterization and lure optimization. Our 3–6 h window is operational guidance derived from behavioral timing and was not chemically validated in this study. Future work should integrate behavioral timing with genotyping, local pheromone characterization, and field evaluation of lures calibrated to the regional early-night schedule.

CONCLUSION

This study demonstrated that the nocturnal mating phase in Indonesian FAW was a consistent among-population signal: mating was concentrated 3–6 h into the scotophase, and timing varied more strongly than mating frequency or success. We therefore propose mating timing as an operational indicator that may inform but cannot diagnose putative strain identity and guide sampling and monitoring decisions. Practically, focusing pheromone-gland extractions within the 3–6 h window is expected to improve comparability of GC–MS analyses and assist rational lure formulation. Any operational use should be paired with genotyping and pheromone chemistry, and validated under field conditions, particularly in low-activity populations, before diagnostic or management claims. Together, behavioral and genetic lines of evidence may support development of more efficient, locally adapted FAW monitoring and control tools.

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AUTHOR DECLARATIONS

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Conflict of Interest

The authors declare that they have no competing interests.

Ethics Declarations

Not applicable. This study did not involve human participants or vertebrate animals requiring ethical approval.

Author's Contributions

VNOS designed and conducted the behavioral observations and collected the data. VNOS and IMS performed the data analysis. VNOS drafted the manuscript, and all authors contributed to the interpretation of results and manuscript revision and approved the final version.

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SUPPLEMENTARY MATERIAL

Table S1. Raw pair-level records for all experimental pairs (mated and unmated) across nine Indonesian populations of *Spodoptera frugiperda*

Population	**Pair_ID**	**Success (0/1)**	**Frequency (count)**	**Duration (h)**	**Precopulatory (d)**	**Male Age (d)**	**Female Age (d)**
LS1	1	0	NA	NA	NA	NA	NA
LS1	2	0	NA	NA	NA	NA	NA
LS1	3	0	NA	NA	NA	NA	NA
LS1	4	1	1	1.5	2.06	2	2
LS1	5	1	2	2.75	1.1	1	1
LS1	6	0	NA	NA	NA	NA	NA
LS1	7	1	1	1	2.06	2	2
LS1	8	0	NA	NA	NA	NA	NA
LS1	9	0	NA	NA	NA	NA	NA
LS1	10	0	NA	NA	NA	NA	NA
LS1	11	0	NA	NA	NA	NA	NA
LS1	12	0	NA	NA	NA	NA	NA
LS1	13	0	NA	NA	NA	NA	NA
LS1	14	1	1	4	3.1	3	4
LS1	15	1	2	4.25	2.17	2	3
LS1	16	1	1	1.5	2.25	2	3
LS1	17	0	NA	NA	NA	NA	NA
LS1	18	0	NA	NA	NA	NA	NA
LS1	19	1	2	0.5	2.1	2	3
LS1	20	0	NA	NA	NA	NA	NA
LS1	21	1	1	0.5	3.02	2	2
LS1	22	1	1	1	2	1	1
LS1	23	1	1	3.5	3.02	2	2

Population	Pair_ID	Success (0/1)	Frequency (count)	Duration (h)	Precopulatory (d)	Male Age (d)	Female Age (d)
LS1	24	0	NA	NA	NA	NA	NA
LS1	25	0	NA	NA	NA	NA	NA
LS2	1	1	4	1.3	0.08	0	0
LS2	2	1	2	1.5	1	1	1
LS2	3	1	4	1.88	1.02	1	1
LS2	4	1	3	1.33	1	1	1
LS2	5	1	1	2	2.08	2	2
LS2	6	1	3	1	0.1	1	1
LS2	7	1	3	1.17	2.02	2	2
LS2	8	0	NA	NA	NA	NA	NA
LS2	9	0	NA	NA	NA	NA	NA
LS2	10	1	1	0.5	4.02	4	5
LS2	11	1	1	2	2.04	1	2
LS2	12	0	NA	NA	NA	NA	NA
LS2	13	0	NA	NA	NA	NA	NA
LS2	14	0	NA	NA	NA	NA	NA
LS2	15	0	NA	NA	NA	NA	NA
LS2	16	1	1	1	2.08	4	4
LS2	17	1	4	0.88	2.02	1	2
LS2	18	0	NA	NA	NA	NA	NA
LS2	19	0	NA	NA	NA	NA	NA
LS2	20	0	NA	NA	NA	NA	NA
LG	1	0	NA	NA	NA	NA	NA
LG	2	1	2	3	4.06	3	3
LG	3	1	4	0.88	1.02	1	1
LG	4	0	NA	NA	NA	NA	NA
LG	5	1	1	1	2	2	2
LG	6	1	2	1.5	2.15	2	2

Population	**Pair_ID**	**Success (0/1)**	**Frequency (count)**	**Duration (h)**	**Precopulatory (d)**	**Male Age (d)**	**Female Age (d)**
LG	7	1	2	1.25	1.06	1	1
LG	8	1	2	0.75	1.17	1	1
LG	9	0	NA	NA	NA	NA	NA
LG	10	1	1	1.5	1	1	3
LG	11	1	4	1.3	0.1	0	1
LG	12	1	3	1.33	1.1	1	1
LG	13	1	3	1.33	1.06	1	1
LG	14	1	3	0.83	1	1	1
LG	15	0	NA	NA	NA	NA	NA
LG	16	0	NA	NA	NA	NA	NA
LG	17	1	2	2.75	0.19	1	4
LG	18	1	2	1.25	1.02	1	5
LG	19	0	NA	NA	NA	NA	NA
LT	1	1	1	1.5	1.04	1	3
LT	2	1	4	1.88	0.19	0	0
LT	3	1	4	0.63	1.08	2	2
LT	4	0	NA	NA	NA	NA	NA
LT	5	0	NA	NA	NA	NA	NA
LT	6	1	1	1	3.04	3	3
LT	7	1	1	0.5	2.04	3	3
LT	8	0	NA	NA	NA	NA	NA
LT	9	0	NA	NA	NA	NA	NA
LT	10	1	1	1	1.04	2	2
LT	11	1	1	2.5	1.04	2	2
LT	12	0	NA	NA	NA	NA	NA
JB	1	1	1	2	1.1	1	1
JB	2	1	4	2	1.04	1	1
JB	3	0	NA	NA	NA	NA	NA

Population	**Pair_ID**	**Success (0/1)**	**Frequency (count)**	**Duration (h)**	**Precopulatory (d)**	**Male Age (d)**	**Female Age (d)**
JB	4	1	1	1	3.19	3	3
JB	5	1	1	0.5	3.19	3	3
JB	6	0	NA	NA	NA	NA	NA
JB	7	0	NA	NA	NA	NA	NA
JB	8	1	1	0.5	2.21	2	3
JB	9	0	NA	NA	NA	NA	NA
JB	10	0	NA	NA	NA	NA	NA
ML	1	0	NA	NA	NA	NA	NA
ML	2	1	2	1	0.1	1	1
ML	3	0	NA	NA	NA	NA	NA
ML	4	1	2	1	1.17	1	1
ML	5	0	NA	NA	NA	NA	NA
ML	6	0	NA	NA	NA	NA	NA
ML	7	0	NA	NA	NA	NA	NA
ML	8	1	1	1.5	2.19	1	2
ML	9	0	NA	NA	NA	NA	NA
ML	10	1	3	2.33	1.13	1	3
ML	11	0	NA	NA	NA	NA	NA
ML	12	0	NA	NA	NA	NA	NA
ML	13	1	1	1.5	1	1	1
ML	14	0	NA	NA	NA	NA	NA
ML	15	1	1	3	2.02	2	2
ML	16	0	NA	NA	NA	NA	NA
ML	17	1	2	2.5	2.02	2	3
ML	18	1	1	1	2.19	4	4
ML	19	0	NA	NA	NA	NA	NA
ML	20	0	NA	NA	NA	NA	NA
ML	21	1	1	2.5	2	1	3

Population	Pair_ID	Success (0/1)	Frequency (count)	Duration (h)	Precopulatory (d)	Male Age (d)	Female Age (d)
ML	22	1	1	2	2	1	2
ML	23	1	1	3.5	4.02	3	5
ML	24	0	NA	NA	NA	NA	NA
ML	25	1	2	3	2	1	3
ML	26	1	1	2	3.1	5	2
ML	27	1	1	1	2.19	1	4
ML	28	1	1	0.5	3.21	1	4
ML	29	0	NA	NA	NA	NA	NA
ML	30	0	NA	NA	NA	NA	NA
KD	1	1	1	1	4	5	5
KD	2	1	2	1.25	3.19	1	2
KD	3	1	1	1	4.02	4	6
KD	4	0	NA	NA	NA	NA	NA
KD	5	1	1	1	4.13	4	5
KD	6	0	NA	NA	NA	NA	NA
KD	7	0	NA	NA	NA	NA	NA
KD	8	1	1	2	1.02	1	2
KD	9	0	NA	NA	NA	NA	NA
KD	10	1	1	1	2.13	2	3
KD	11	0	NA	NA	NA	NA	NA
KD	12	1	1	1.5	1.04	1	2
KD	13	0	NA	NA	NA	NA	NA
KD	14	1	2	1.5	2.06	2	3
KD	15	1	3	1	2.17	2	3
KD	16	1	1	1	2.23	2	1
KD	17	0	NA	NA	NA	NA	NA
KD	18	1	1	2	5	5	6
KD	19	1	2	0.5	3.15	3	4

Population	Pair_ID	Success (0/1)	Frequency (count)	Duration (h)	Precopulatory (d)	Male Age (d)	Female Age (d)
KD	20	1	1	2	2.02	2	4
BW	1	0	NA	NA	NA	NA	NA
BW	2	1	1	3	2.02	2	3
BW	3	0	NA	NA	NA	NA	NA
BW	4	0	NA	NA	NA	NA	NA
BW	5	0	NA	NA	NA	NA	NA
BW	6	1	2	1	1.08	1	1
BW	7	0	NA	NA	NA	NA	NA
BW	8	0	NA	NA	NA	NA	NA
BW	9	1	2	1	2.23	2	3
BW	10	1	1	1	2.17	2	3
BW	11	1	1	1	1.04	1	1
BW	12	0	NA	NA	NA	NA	NA
BW	13	1	2	1	1.06	1	1
BW	14	0	NA	NA	NA	NA	NA
BW	15	1	2	1	1.17	1	1
BW	16	1	1	0.5	1.17	2	3
SP	1	0	NA	NA	NA	NA	NA
SP	2	0	NA	NA	NA	NA	NA
SP	3	0	NA	NA	NA	NA	NA
SP	4	0	NA	NA	NA	NA	NA
SP	5	1	1	0.5	3.23	4	4
SP	6	1	1	0.5	2.25	2	2
SP	7	0	NA	NA	NA	NA	NA
SP	8	0	NA	NA	NA	NA	NA
SP	9	0	NA	NA	NA	NA	NA
SP	10	1	2	2.25	1.02	2	2
SP	11	1	4	1.5	0.13	1	2

Population	Pair_ID	Success (0/1)	Frequency (count)	Duration (h)	Precopulatory (d)	Male Age (d)	Female Age (d)
SP	12	1	1	1.5	1.08	2	3

Variables include population code, pair identifier, mating success (0/1), copulation frequency (count), copulation duration (h), precopulatory period (days), and male and female age at mating (days). Only first-event onsets are reported for timing analyses; non-mated pairs are indicated as NA for onset and duration.

Table S2. First-event copulation onset times for mated pairs used in circular analyses

Population	**Pair_ID**	**First-event Onset (h)**
LS1	4	2
LS1	5	2.5
LS1	7	2.5
LS1	14	3.5
LS1	15	3.5
LS1	16	4.5
LS1	19	4.5
LS1	21	4.5
LS1	22	6
LS1	23	8
LS2	1	2
LS2	2	2
LS2	3	2.5
LS2	4	2.5
LS2	5	2.5
LS2	6	2.5
LS2	7	3
LS2	10	4
LS2	11	4
LS2	16	4.5
LS2	17	5.5
LG	2	2
LG	3	2
LG	5	2
LG	6	2.5
LG	7	2.5
LG	8	3.5
LG	10	3.5
LG	11	4.5
LG	12	4.5
LG	13	5.5
LG	14	6
LG	17	6
LG	18	6.5
LT	1	3
LT	2	3
LT	3	3
LT	6	3.5
LT	7	3.5
LT	10	4
LT	11	6.5
JB	1	3
JB	2	5.5
JB	4	6.5
JB	5	6.5

Population	Pair_ID	First-event Onset (h)
JB	8	7
ML	2	2
ML	4	2
ML	8	2
ML	10	2
ML	13	2.5
ML	15	2.5
ML	17	2.5
ML	18	4.5
ML	21	4.5
ML	22	5
ML	23	6
ML	25	6
ML	26	6
ML	27	6.5
ML	28	7
KD	1	2
KD	2	2.5
KD	3	2.5
KD	5	2.5
KD	8	3
KD	10	3.5
KD	12	4.5
KD	14	5
KD	15	5
KD	16	5.5
KD	18	6
KD	19	6.5
KD	20	7.5
BW	2	2.5
BW	6	4
BW	9	4.5
BW	10	5
BW	11	6
BW	13	6
BW	15	6
BW	16	7.5
SP	5	2.5
SP	6	3.5
SP	10	4
SP	11	7.5
SP	12	10

First-event copulation onset is expressed as hours since lights-off. Only one row per mated pair is included to preserve statistical independence in timing analyses. Valid range: onset_h ∈ [0,12].

Table S3. Pairwise contrasts and estimated marginal means for copulation frequency from the negative-binomial GLM

Contrast	**Estimate_Ratio**	**Lower CL**	**Upper CL**	**SE**	**df**	**Stat**	***P*-value**	**Interpretation**
LS1 / BW	0.6933	0.3829	1.2554	0.3029	inf	-0.8383	0.9957	Not significant
LS1 / JB	0.6500	0.3465	1.2192	0.3209	inf	-0.8726	0.9943	Not significant
LS1 / KD	0.5778	0.3677	0.9080	0.2306	inf	-1.3742	0.9077	Not significant
LS1 / LG	0.3187	0.2530	0.4016	0.1179	inf	-3.0912	0.0516	Marginal
LS1 / LS2	0.3852	0.2902	0.5113	0.1446	inf	-2.5422	0.2118	Not significant
LS1 / LT	0.4800	0.3183	0.7239	0.2096	inf	-1.6809	0.7585	Not significant
LS1 / ML	0.7429	0.4255	1.2968	0.2843	inf	-0.7768	0.9975	Not significant
LS1 / SP	0.6933	0.3640	1.3207	0.3288	inf	-0.7724	0.9976	Not significant
BW / JB	0.9375	0.3694	2.3791	0.4751	inf	-0.1273	1.0000	Not significant
BW / KD	0.8333	0.4229	1.6422	0.3461	inf	-0.4390	1.0000	Not significant
BW / LG	0.4597	0.3243	0.6516	0.1780	inf	-2.0070	0.5383	Not significant
BW / LS2	0.5556	0.3624	0.8517	0.2180	inf	-1.4980	0.8569	Not significant
BW / LT	0.6923	0.3752	1.2774	0.3125	inf	-0.8146	0.9965	Not significant
BW / ML	1.0714	0.4631	2.4790	0.4280	inf	0.1727	1.0000	Not significant
BW / SP	1.0000	0.3844	2.6017	0.4878	inf	0.0000	1.0000	Not significant
JB / KD	0.8889	0.3888	2.0325	0.4220	inf	-0.2481	1.0000	Not significant
JB / LG	0.4903	0.3181	0.7559	0.2208	inf	-1.5825	0.8147	Not significant
JB / LS2	0.5926	0.3494	1.0050	0.2695	inf	-1.1506	0.9663	Not significant
JB / LT	0.7385	0.3547	1.5374	0.3741	inf	-0.5985	0.9996	Not significant
JB / ML	1.1429	0.4070	3.2090	0.5267	inf	0.2897	1.0000	Not significant
JB / SP	1.0667	0.3454	3.2938	0.5753	inf	0.1197	1.0000	Not significant
KD / LG	0.5516	0.3802	0.8003	0.1899	inf	-1.7285	0.7290	Not significant
KD / LS2	0.6667	0.4220	1.0531	0.2333	inf	-1.1587	0.9649	Not significant
KD / LT	0.8308	0.4226	1.6331	0.3448	inf	-0.4467	1.0000	Not significant
KD / ML	1.2857	0.5218	3.1680	0.4601	inf	0.7023	0.9988	Not significant
KD / SP	1.2000	0.4121	3.4940	0.5453	inf	0.4012	1.0000	Not significant
LG / LS2	1.2086	0.5715	2.5556	0.3821	inf	0.5993	0.9996	Not significant
LG / LT	1.5061	0.4805	4.7205	0.5829	inf	1.0581	0.9799	Not significant

Contrast	**Estimate_Ratio**	**Lower CL**	**Upper CL**	**SE**	**df**	**Stat**	***P*-value**	**Interpretation**
LG / ML	2.3308	0.5284	10.2819	0.7572	inf	2.6048	0.1844	Not significant
LG / SP	2.1754	0.3494	13.5453	0.9331	inf	1.8121	0.6742	Not significant
LS2 / LT	1.2462	0.4782	3.2473	0.4887	inf	0.5612	0.9998	Not significant
LS2 / ML	1.9286	0.5520	6.7384	0.6383	inf	1.9844	0.5543	Not significant
LS2 / SP	1.8000	0.3899	8.3088	0.7804	inf	1.3558	0.9141	Not significant
LT / ML	1.5476	0.4611	5.1949	0.6178	inf	1.0939	0.9753	Not significant
LT / SP	1.4444	0.3632	5.7448	0.7044	inf	0.7541	0.9980	Not significant
ML / SP	0.9333	0.4174	2.0872	0.4106	inf	-0.1568	1.0000	Not significant

Entries are presented as incidence-rate ratios (IRR) with 95% confidence intervals and exact *P*-values. The default reference level is LS1 unless otherwise stated. Model family: negative-binomial with log link and offset for unequal interval durations.

Table S4. Population-level frequency summaries for copulation frequency by population

Population	**Mean Frequency (Count)**	**Proportion of Zeros**	**n (pairs)**
LS1	0.52	0.60	25
LS2	1.35	0.45	20
LG	1.63	0.32	19
LT	1.08	0.42	12
JB	0.80	0.50	10
ML	0.70	0.50	30
KD	0.90	0.35	20
BW	0.75	0.50	16
SP	0.75	0.58	12

Population-level summaries include mean copulation frequency, proportion of zeros, and number of pairs for each population. Full model diagnostics are reported in the Table 3 footnote of the main manuscript.

Table S5. Monte Carlo sensitivity estimates for Rayleigh's test by population

Population	**N (Mated Pairs)**	**r**	**Rayleigh Sensitivity**	**95% MC CI**
LS2	11	0.847	1.000	0.999–1.000
LT	7	0.841	0.983	0.979–0.986
LG	13	0.674	0.964	0.959–0.969
KD	13	0.648	0.941	0.934–0.947
BW	8	0.749	0.914	0.906–0.922
ML	15	0.579	0.899	0.890–0.907
LS1	10	0.675	0.893	0.884–0.902
JB	5	0.754	0.700	0.688–0.713
SP	5	0.283	0.109	0.101–0.118

Monte Carlo sensitivity (5,000 replicates; $\alpha = 0.05$) was estimated using the observed sample size (n) and mean resultant length (r) for each population. Sensitivity values represent expected statistical power under the observed conditions and support interpretation of non-significant results in populations with small sample sizes or weak concentration.