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Multiple lines of evidence for sex-biased dispersal in the nocturnal pitviper *Viridovipera stejnegeri*

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ABSTRACT

Sex-biased dispersal, a widespread phenomenon in the animal kingdom, plays a crucial role in shaping genetic structure, optimizing population dynamics, and sustaining reproductive success. Recent research has uncovered considerable diversity and complexity in sex-biased dispersal patterns across species, although studies on snakes with secretive behaviors and low encounter rates remain limited. In this study, sex-biased dispersal in the Asian pitviper (*Viridovipera stejnegeri*) was investigated using microsatellite markers and a mark-recapture approach. The underlying factors contributing to sex-biased dispersal were also examined, with a focus on genetic structure, kinship patterns, and resource competition. Microsatellite analyses revealed an overall pattern of female-biased dispersal in *V. stejnegeri*, although certain populations exhibited male-biased dispersal. Notably, these variations in dispersal patterns among populations were not associated with genetic differentiation. The contrasting results between microsatellite data and mark-recapture methods highlighted the limitations of using mark-recapture alone to investigate sex-biased dispersal in snakes. Analyses indicated no significant differences in intrasexual and intersexual resource competition pressures, thus failing to support the resource competition hypothesis. Kinship analysis showed no significant clustering of consanguineous individuals within subpopulations; rather, individuals dispersed into neighboring subpopulations through sex-biased dispersal, effectively reducing the risk of inbreeding and supporting the inbreeding avoidance hypothesis. However, further investigation is needed to determine whether the local mate competition hypothesis applies. Overall, this study provides valuable insights into methodological approaches and data interpretation relevant to biodiversity conservation and management strategies.

Keywords: Sex-biased dispersal; Genetic differentiation; Kinship; Resource competition; Snake

INTRODUCTION

Sex-biased dispersal occurs when differential reproductive or survival costs and benefits between sexes drive one sex to preferentially disperse, while the other tends to remain in the natal population. This divergence exposes each sex to distinct evolutionary forces, producing dispersal asymmetry in which one sex tends to emigrate while the other remains philopatric—a pattern defined as sex-biased dispersal (Yu et al., 2022). This phenomenon is a key factor in the establishment of fine-scale genetic structures within populations, facilitating local adaptation, and influencing broader evolutionary processes, population dynamics, and species persistence (Guerrini et al., 2014).

While the study of dispersal patterns and mechanisms in animals has garnered increased attention in recent years, the focus is heavily skewed towards mammals and birds (Yu et al., 2022), with birds (mostly monogamous) predominantly exhibiting female-biased dispersal and mammals (mostly polygynous) primarily exhibiting male-biased dispersal (Greenwood, 1980; Nemesházi et al., 2018). In contrast, research on sex-biased dispersal in snakes is limited and has produced diverse and sometimes conflicting results. Notably, while male-biased dispersal has been reported as the dominant pattern in various snake species (Dubey et al., 2008; Folt et al., 2019; Keogh et al., 2007; Lane & Shine 2011; Lukoschek et al., 2008; Pernetta et al., 2011; Wen et al., 2021; Zwahlen et al., 2021), cases of female-biased dispersal (Lane & Shine, 2011; Rivas & Burghardt, 2005; Yu et al., 2022) and unbiased dispersal (Lukoschek et al., 2008) have also been documented. Furthermore, even within the same species,

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disparate dispersal patterns have been detected across populations (Lukoschek et al., 2008; Zwahlen et al., 2021), further complicating our understanding of dispersal dynamics in snakes. These findings underscore the need for more comprehensive research to elucidate the mechanisms and evolutionary drivers underlying sex-biased dispersal in snake populations.

The mechanisms driving sex-biased dispersal have long been debated, and various hypotheses have been proposed to explain this phenomenon (Clutton-Brock & Lukas, 2012). For instance, Schweizer et al. (2007) linked sex-biased dispersal to differences in reproductive success between dispersing and non-dispersing individuals, while Trochet et al. (2016) emphasized the role of sexual dimorphism in morphology and parental care, arguing that these factors, rather than mating systems, are the primary determinants of dispersal patterns. The most widely recognized explanations for sex-biased dispersal include the inbreeding avoidance hypothesis, local mate competition hypothesis, and resource competition hypothesis (Dobson & Jones, 1985; Moore & Ali, 1984; Waser et al., 1986). The inbreeding avoidance hypothesis posits that one sex disperses to reduce the likelihood of inbreeding and its associated fitness costs (Vigilant et al., 2015). The resource competition hypothesis suggests that intrasexual or intersexual competition for resources within a population leads to the dispersal of either males or females as a means to alleviate competition pressure (Greenwood, 1980). The local mate competition hypothesis asserts that individuals of the sex experiencing stronger reproductive selection pressure are more likely to disperse in pursuit of increased mating opportunities (Dobson, 1982). However, it is now widely accepted that no single hypothesis can fully explain the complex mechanisms driving sex-biased dispersal, and that multiple factors often interact to shape these patterns.

The nocturnal and arboreal Stejneger's bamboo pitviper (*Viridovipera stejnegeri*), a venomous species broadly distributed across southern China and Vietnam (Guo et al., 2021), provides an ideal model for exploring sex-biased dispersal due to its extensive geographic range and frequent encounters in the field. Although male-biased dispersal predominates in most snake taxa (Yu et al., 2022), the extent and drivers of sex-specific dispersal in this species remain poorly characterized. Therefore, to evaluate the hypothesis that males are more prone to dispersal, this study employed an integrative approach combining microsatellite-based genetic inference with mark-recapture field data. In parallel, potential ecological and demographic determinants of sex-biased dispersal were assessed, including kinship structure and intersexual variation in resource use. This study aimed to elucidate the mechanisms governing sex-biased dispersal in *V. stejnegeri* and inform broader understanding of dispersal strategies in secretive reptilian taxa.

MATERIALS AND METHODS

Sampling and microsatellite amplification

A total of 95 *V. stejnegeri* individuals (53 males, 42 females) were sampled from four distinct populations across China, including Sichuan (SC), Yunnan (YN), Anhui (AH), and Jiangxi (JX) (Figure 1). Sampling was conducted under permits from local forestry authorities. After euthanization, fresh liver tissue was collected and preserved in 95% ethanol for subsequent DNA extraction and genetic analyses. Detailed sample information is provided in Supplementary Table S1.

Genomic DNA was extracted using the TIANmap Genomic DNA Kit (Tiangen Biotech, China). Fourteen microsatellite loci and primers developed by Chang et al. (2016) for *V. stejnegeri* were selected for polymerase chain reaction (PCR) amplification to assess sex-biased dispersal and genetic

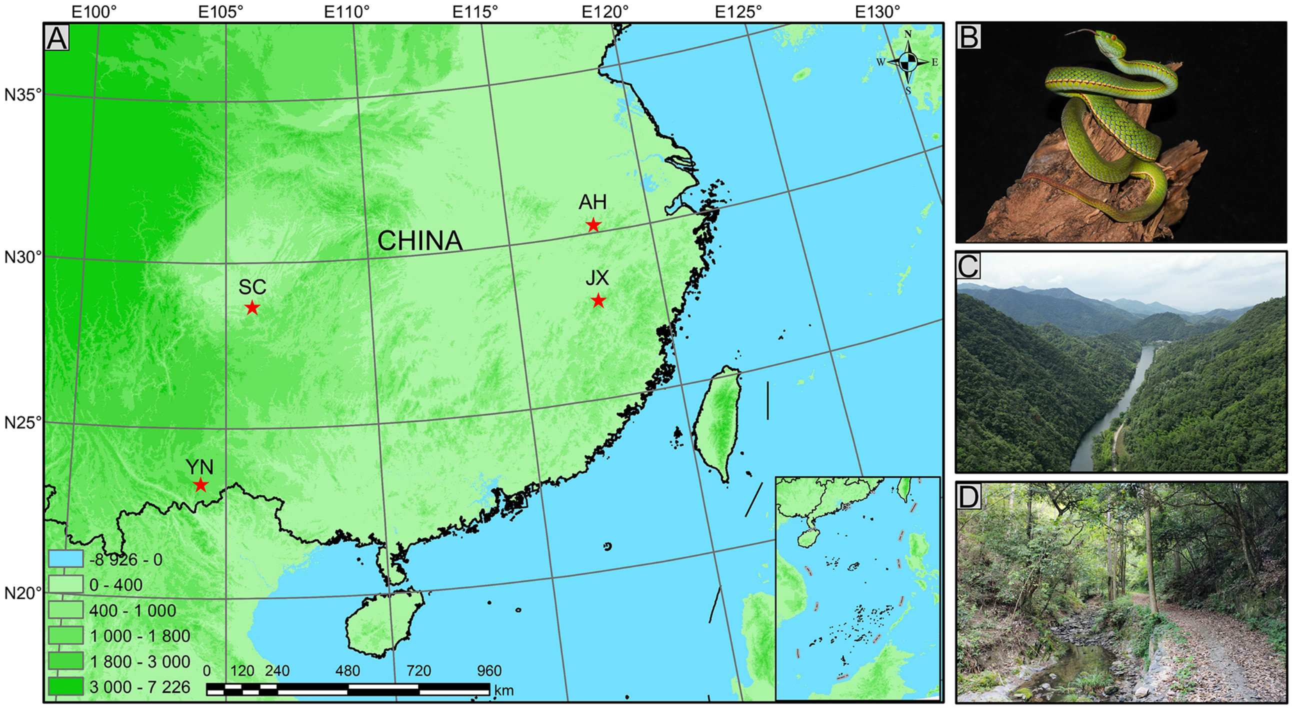

Figure 1 Sampling localities and habitat characteristics of *Viridovipera stejnegeri*

A: Geographic distribution of sampling sites, indicated by red pentagons. B: Overview of typical landscape. C: Representative landscape-scale habitat. D: Typical microhabitat. All habitat photographs were taken in Huangshan, Anhui, China by S.W. Tan.

differentiation. Detailed information on microsatellite loci and primers is provided in Supplementary Table S2.

PCR was performed in a total volume of 25 µL, consisting of 1 µL of DNA, 1 µL of each forward and reverse primer (with the forward primer labeled with a fluorescent marker, 10 µmol/L), 12.5 µL of 2×T5 Super PCR Mix (PAGE) (Beijing Tsingke Biotech, China), and 9.5 µL of nuclease-free water. The amplification protocol involved an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 20 s, annealing at 55–65°C for 30 s, extension at 72°C for 30 s, and post-extension at 72°C for 7 min. PCR products were first checked for quality using agarose gel electrophoresis, and high-quality products were then subjected to capillary electrophoresis. Fluorescently labeled forward primers of each product were measured using an ABI 3730xl DNA Analyzer (Applied Biosystems, USA), and measurements were analyzed using Geneious Prime v.2023.2.1, with each sample read at least three times to ensure accuracy (Yu et al., 2022). Micro-Checker v.2.2.3 (Van Oosterhout et al., 2004) and FreeNA (Chapuis & Estoup, 2007) were used to detect null alleles, stuttering, large allele dropout error, and other anomalies in the data (Wen et al., 2013).

Analysis of sex-biased dispersal based on microsatellite data

To assess sex-biased dispersal, several genetic indices, including genetic differentiation (F_{st}), inbreeding coefficient (F_{is}), gene diversity (H_s), relatedness (r), mean assignment index (mAIc), and variance of assignment index (vAIc), were calculated in FSTAT v.2.9.3 (Goudet et al., 2002; Wang et al., 2019; Yu et al., 2022). Significant genetic differentiation between eastern and western populations of *V. stejnegeri* has been previously reported (Guo et al., 2016). Accordingly, analyses were conducted across all four populations as a whole, as well as separately for the eastern and western populations. To further assess patterns of sex-biased dispersal, r values between males and females were compared using COANCESTRY v.1.0 (Wang, 2011).

Genetic differentiation analyses

Genetic distances between individual samples were calculated based on microsatellite data using the GenAlEx v.6.5 add-in for Excel (Peakall & Smouse, 2012) and subsequently used to construct a neighbor-joining (NJ) phylogenetic tree in MEGA v.11 (Tamura et al., 2021). The resulting tree was further annotated and visually enhanced, with groupings added using the ChiPlot online platform (https://www.chiplot.online/; Xie et al., 2023). To investigate genetic structure, STRUCTURE v.2.3.4 was used to analyze clustering patterns across the four populations (Pritchard et al., 2000). The optimal number of genetic clusters (K) was determined using the Delta K method via the StructureSelector online tool (https://lmme.ac.cn/StructureSelector/; Li & Liu, 2018). CLUMPP v.1.1.2 was then used to integrate the results of 10 independent runs corresponding to the optimal K value with 10 000 random permutations, followed by resampling to analyze the most likely number of genetic clusters (Jakobsson & Rosenberg, 2007). Distruct v.1.1 was used to graphically depict the population structure (Rosenberg, 2004). Additionally, discriminant analysis of principal components (DAPC) was conducted using the "adegenet" package in R to further explore the patterns of genetic differentiation (Jombart et al., 2010).

Kinship analyses

Kinship relationships were examined within each population and among the three AH subpopulations to examine intra- and intergroup genetic structure using KINGROUP v.2 (Konovalov et al., 2004). The dataset was first converted into a compatible "kinship" format using the GenAlEx v.6.5 add-in for Excel (Peakall & Smouse, 2012) and subsequently imported into KINGROUP v.2 for further analysis. Parentage analysis was conducted using the "(Rm, Rp)-coefficients" method under the "Likelihood" section in the "Pedigree" menu, followed by the calculation of sibling and half-sibling relationships via the "Descending Ratio" method in the "FSR" menu. Relationship network diagrams were then generated using the ChiPlot online platform (https://www.chiplot.online/).

To assess whether individuals within the same subpopulation were genetically more related, pairwise average relatedness both within and between subpopulations was calculated using KINGROUP v.2, analyzing males and females collectively and separately (Wang et al., 2021).

An isolation-by-distance (IBD) pattern analysis was performed using the "mantel" function from the R package "vegan" to examine the correlation between individual genetic relationships and geographical distances among the three subpopulations (Oksanen et al., 2025). Genetic and geographical distances were calculated using GenAlEx v.6.5, and the significance of their correlation was tested using a Mantel test with 1 000 permutations (Wang et al., 2021).

Mark-recapture and habitat data collection

To test the hypothesis that resource competition drives dispersal, both habitat and mark-recapture data were collected from the AH population (Figure 1), which consists of three adjacent subpopulations: Tangjiazhuang (AH-T: E118°01′01″, N30°13′39″), Duduken (AH-D: E118°01′33″, N30°14′28″), and Luken (AH-L: E118°01′39″, N30°16′02″). These subpopulations are distributed along three parallel streams and separated by intervening hillslopes with distances ranging from 1 km to 10 km.

Field surveys were conducted during four survey periods: June–July 2022, September–October 2022, June–July 2023, and September–October 2023. All surveys were carried out at night between 2000h and 0200h. During each session, individuals from the three subpopulations were located, and their counts, geographical coordinates (latitude and longitude), and sex were recorded. Individuals were uniquely marked via ventral scale clipping and tail tip notching for subsequent identification. To evaluate the sex-biased dispersal model, post-breeding season recapture rates were compared between sexes following previously established protocols (Huang et al., 2015), with differences analyzed using chi-square tests. As no juveniles were detected during the field survey, only reproductive dispersal was assessed. At each presence site, 4 m×4 m quadrats were established (Strine et al., 2018), and 13 habitat variables were recorded. Details of these variables and measurement protocols are summarized in Supplementary Table S3.

To measure dispersal distances, electronic tags (EM4305; wnLikes, China) were implanted subcutaneously beneath the dorsal surface of each individual in June 2023. These tags are non-invasive and do not adversely affect survival or behavior. In September 2023, an extensive search for individuals with electronic tags was conducted, and their precise locations were georeferenced using GPS. Dispersal distances were calculated using Omap v.9.9. Mean dispersal distances by sex were computed, and the Mann-Whitney *U* test was used to determine significant differences between male and female dispersal distances.

Habitat competition analysis

Habitat selection preferences of male and female *V. stejnegeri* were quantified using the Vanderploeg (W_i) and Scavia (E_i) selection indices (Vanderploeg & Scavia, 1979):

$$W_i = \left(\frac{r_i}{p_i}\right) / \sum\left(\frac{r_i}{p_i}\right) \quad (1)$$

$$E_i = (W_i - \frac{1}{n})/(W_i + \frac{1}{n}) \quad (2)$$

Where r_i denotes the number of quadrats selected by *V. stejnegeri* with characteristic *i*; p_i denotes the total number of quadrats in the environment with characteristic *i*; and *n* refers to the level or category number of a particular habitat factor. E_i ranges from −1 to 1, with $E_i=1$ indicating strong preference, $0.1<E_i<1$ indicating selection, $-0.1<E_i<0.1$ indicating random selection, $-1<E_i<-0.1$ indicating avoidance, and $E_i=-1$ indicating no selection.

To assess significant differences in habitat selection between males and females, principal component analysis (PCA) and permutational multivariate analysis of variance (PERMANOVA; ADONIS) were performed to analyze habitat similarity. Additionally, analysis of similarities (ANOSIM) was conducted using the "vegan" package in R to assess habitat similarity within and between males and females. Pairwise similarity coefficient matrices, based on cosine similarity, were computed using SPSS v.27, from which average similarity coefficients within and between sexes were calculated. Higher similarity coefficients reflect increased intrasexual habitat overlap and potential competition among individuals.

Analyses not explicitly described above were conducted in R v.4.2.3 and SPSS v.27. Unless otherwise noted, graphs were plotted using R v4.2.3 (R Core Team, 2018) and Origin 2022 (OriginLab, USA). All analyses were conducted with a confidence level of 0.05.

RESULTS

Sex-biased dispersal based on microsatellite data

Microsatellite data analyzed with FSTAT v.2.9.3 revealed distinct sex-specific dispersal patterns. When all individuals were pooled, females exhibited lower values of F_{st}, mAIc, and *r* but higher values of F_{is}, H_{s}, and vAIc compared to males (Table 1), consistent with female-biased dispersal. This overall trend was also observed when the eastern and western populations were analyzed separately, although results for the western population were not statistically significant ($P>0.05$). Further single-population comparisons showed that females had lower *r* values than males in the AH, JX, and YN populations, again suggesting higher female dispersal. In contrast, males in the SC population displayed lower relatedness, indicating male-biased dispersal in this population (Table 2).

Genetic differentiation

The NJ phylogenetic tree constructed using microsatellite data indicated that the four populations grouped into three distinct genetic clusters. The SC and YN populations formed one cluster, while the AH and JX populations each constituted separate clusters (Figure 2). Population structure analysis indicated that the optimal number of genetic clusters was three, with the highest Delta *K* value observed at *K*=3, further supporting the presence of three genetic groups across populations (Figure 3). Further DAPC analysis corroborated these findings, with Linear Discriminant 1 (LD1) and Linear Discriminant 2 (LD2) successfully distinguishing the four populations into the same genetic clusters, consistent with both the NJ tree and STRUCTURE analyses (Figure 4).

Kinship analyses

Kinship analysis within populations identified a substantial presence of consanguineous individuals, with half-sibling relationships far exceeding full-sibling relationships (48.57% vs. 5.71%; Figure 5A). Within the AH population, which includes three subpopulations, consanguineous pairings were detected across all groups. Notably, the intermediate subpopulation harbored the highest proportion of kin-related individuals (52% vs. 73.33% vs. 41.18%). Comparative analysis of kinship patterns revealed that intra- and inter-subpopulation pairings exhibited a higher overall proportion of consanguinity than intrapopulation pairings (52.83% vs. 43.40%; Figure 5B), suggesting stronger genetic connectivity across subpopulations than within each local group.

Pairwise analyses of average relatedness revealed no significant differences among males, females, and the full sample, regardless of whether comparisons were made within subpopulations, between subpopulations, or across all subpopulations (Figure 6). Similarly, IBD analysis showed no significant correlation between genetic distance and geographical distance ($r=0.0675$, $P=0.101$; Figure 7), indicating that geographic separation exerts limited influence on genetic structuring in this species.

Sex-biased dispersal based on mark-recapture

The mark-recapture data from the AH population (Figure 8A)

Table 1 Sex-biased dispersal analysis of all snakes and east and west populations based on FSTAT

Population	Sex	F_{st}	F_{is}	H_s	mAIc	vAIc	*r*
All snakes	M	0.1158	0.1469	0.8467	3.4394	29.6461	0.1859
	F	0.0669	0.1748	0.9079	–4.5300	151.6949	0.1089
	P-value	0.0011	0.3392	0.0008	0.0002	0.0004	0.0015
East population	M	0.1403	0.1469	0.8136	4.8099	24.4277	0.2215
	F	0.0700	0.2240	0.8926	–6.7695	198.7025	0.1095
	P-value	0.0006	0.0294	0.0004	0.0002	0.0004	0.0006
West population	M	0.0279	0.1542	0.9294	0.1845	26.9841	0.0474
	F	0.0223	0.0877	0.9331	–0.2108	32.7093	0.0403
	P-value	0.7315	0.1870	0.8460	0.8475	0.6279	0.8039

Abbreviations are explained in the main text. M: Male; F: Female.

Table 2 Single-population analysis of sex-biased dispersal based on COANCESTRY

Sex	Overall	SC	YN	JX	AH
M	0.0078	–0.0909	0.0616	–0.0155	0.1772
F	–0.0393	0.0052	0.0125	–0.0285	0.0455

Values represent relatedness (*r*) estimated using Wang's method in COANCESTRY. M: Male; F: Female. Four populations included: Sichuan (SC), Yunnan (YN), Anhui (AH), and Jiangxi (JX).

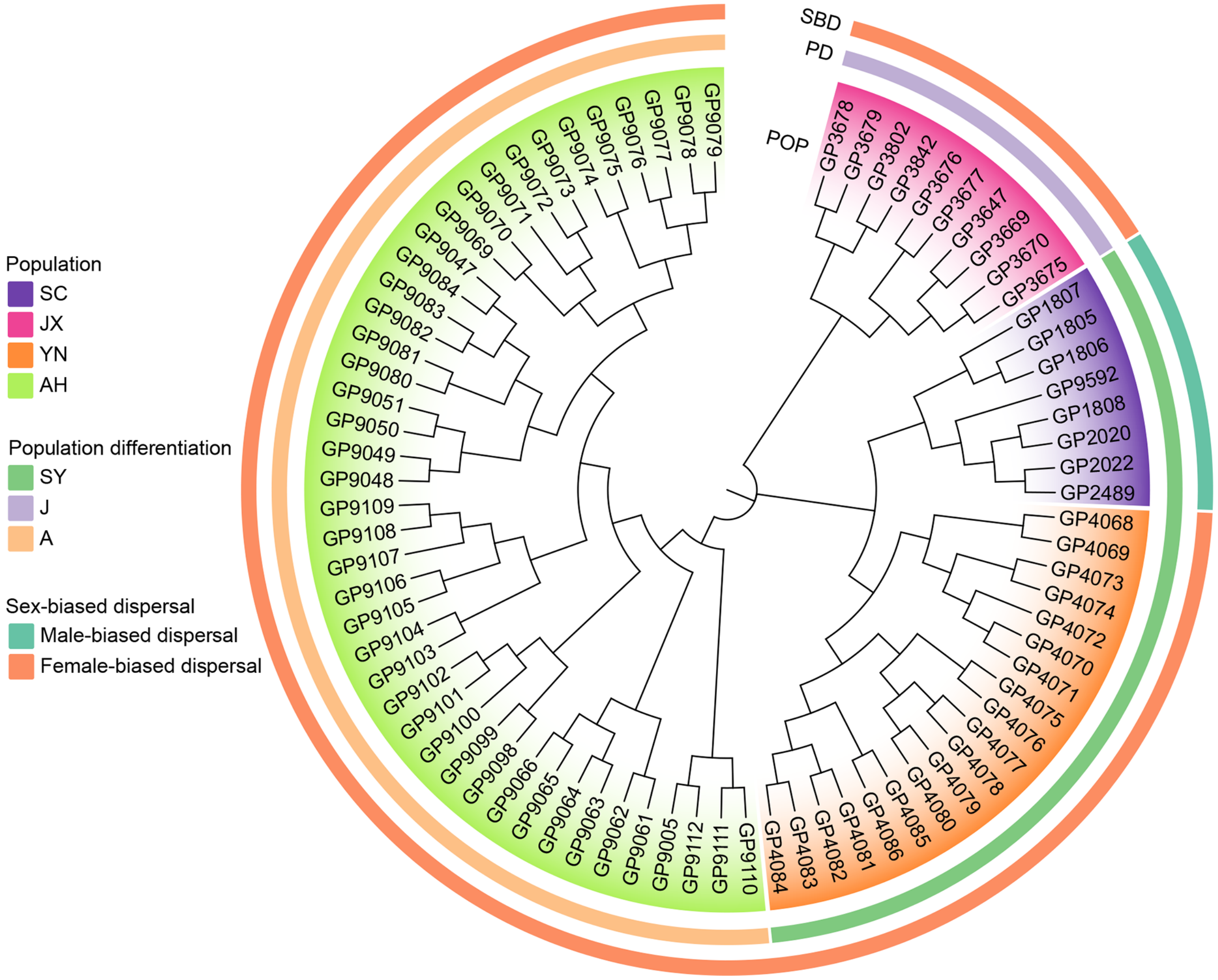

Figure 2 Neighbor-joining (NJ) phylogenetic tree of *Viridovipera stejnegeri* based on microsatellite data

POP, population; PD, population differentiation; SBD, sex-biased dispersal.

revealed no significant differences in the post-breeding recapture rates between females and males (30.8% vs. 26.7%, *P*=0.837). In contrast, females from the AH population exhibited a markedly greater mean dispersal distance than males (416.25 m vs. 142 m, *P*=0.016; Figure 8B).

Habitat selection and competition

Habitat preference analysis based on the Vanderploeg (W_i) and Scavia (E_i) indices indicated broadly similar selection patterns between males (*n*=131) and females (*n*=46) for most key environmental variables, including altitude, temperature, humidity, vegetation coverage, slope, slope position, distance from roads, distance from residential sites, landscape habitat, and vegetation type (Figure 9). However, sex-specific differences emerged in relation to vegetation height, distance from water, and aspect.

The PCA results (Figure 10A) revealed no significant differences in habitat selection between males and females, a finding corroborated by ADONIS analysis (Figure 10B), which showed high within- and between-sex habitat similarity without statistical significance (*P*=0.139; Figure 10C). These results were further supported by cosine similarity metrics from pairwise comparisons, with average coefficients indicating minimal variation in habitat use within males (0.941), within females (0.961), and between sexes (0.939) (Figure 10D).

DISCUSSION

Sex-biased dispersal of *V. stejnegeri*

Although numerous methods have been employed to investigate sex-biased dispersal in snakes, studies combining multiple analytical approaches remain rare (Keogh et al., 2007). Moreover, previous findings have often been inconsistent, reflecting the complexity and diversity of dispersal dynamics across taxa. In this study, microsatellite-based genetic analyses were integrated with mark-recapture data to provide a comprehensive assessment of sex-biased

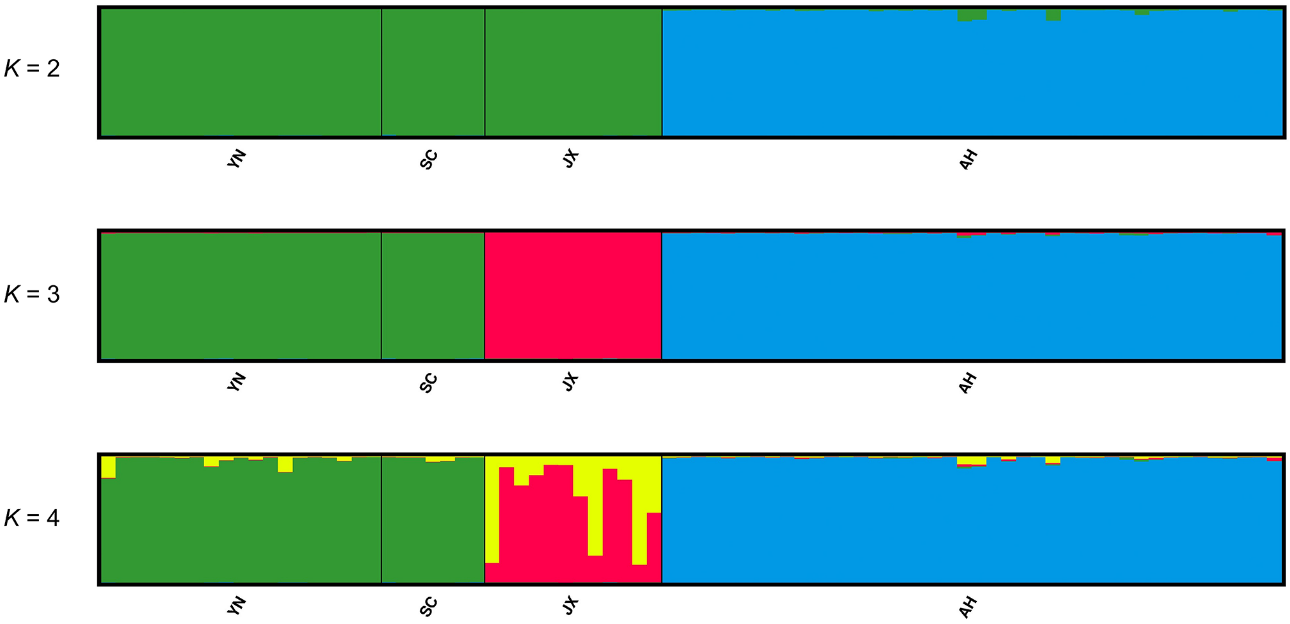

Figure 3 Genetic structure of *Viridovipera stejnegeri* inferred from STRUCTURE analysis at *K*=2–4.

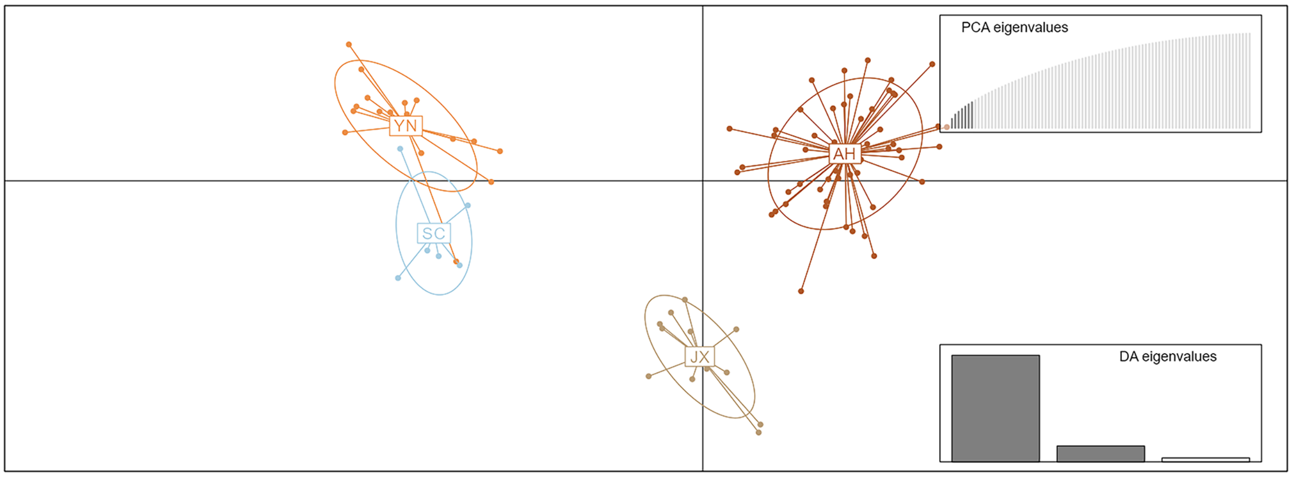

Figure 4 Discriminant analysis of principal components (DAPC) of *Viridovipera stejnegeri* populations

Scatter plot showing the first two linear discriminants (LD). Each dot represents an individual; clusters are denoted by circles; letters indicate population identities.

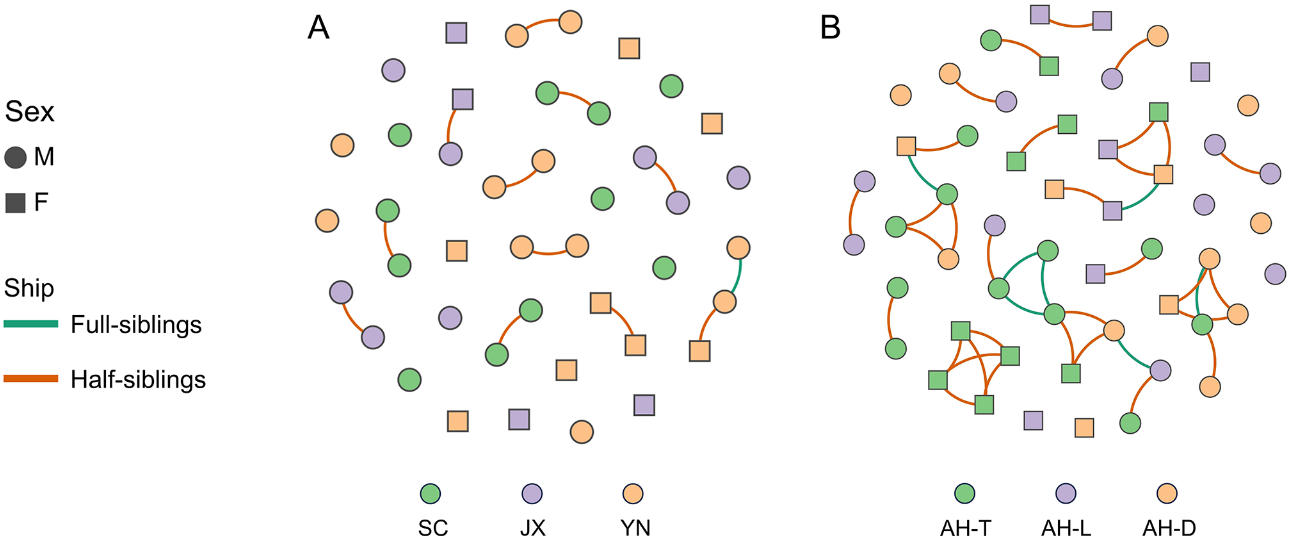

Figure 5 Kinship analysis within and among subpopulations of the AH population of *Viridovipera stejnegeri*

A: Intrapopulation kinship. B: Inter-subpopulation kinship. M: Male; F: Female.

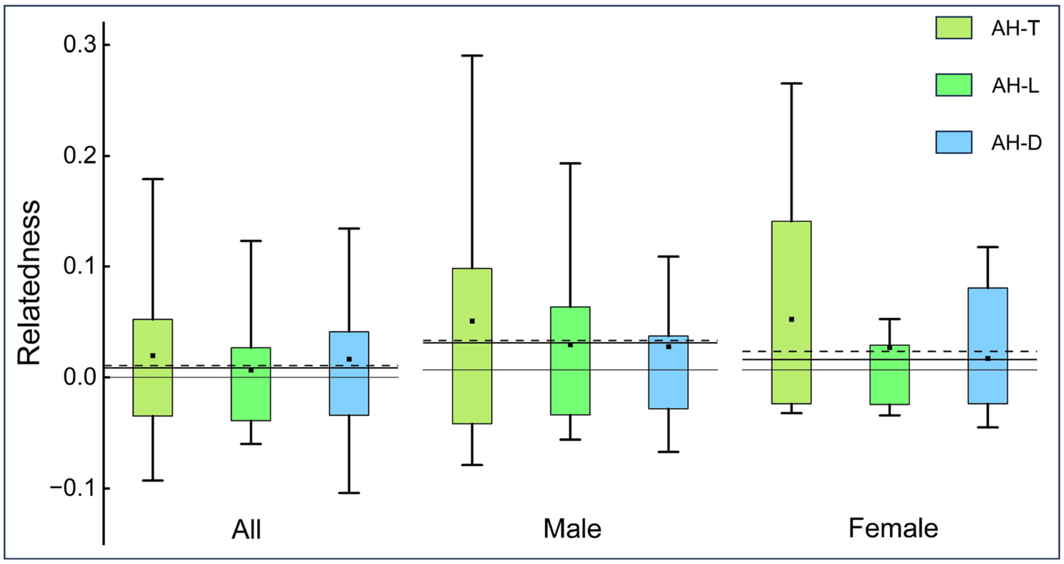

Figure 6 Average coefficient of relatedness (*R*) between individual pairs within and between AH subpopulations

Solid line represents average coefficient of relatedness between subpopulations; dotted line represents average coefficient of relatedness between pairs of individuals from all AH subpopulations.

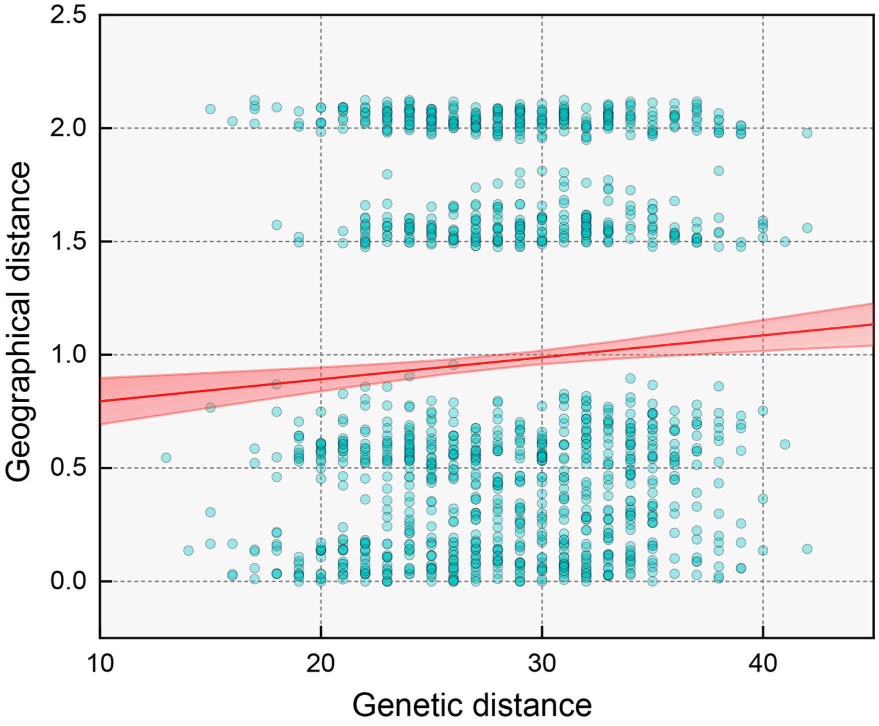

Figure 7 Relationship between genetic and geographic distance based on IBD analysis of individual pairs (AH population)

dispersal in *V. stejnegeri*.

Prior microsatellite-based studies have indicated that dispersing individuals tend to exhibit higher inbreeding coefficients (F_{is}), gene diversity (H_{s}), and variance in assignment index (vAIc), alongside lower mean assignment index (mAIc), genetic differentiation (F_{st}), and r, relative to philopatric individuals (Wang et al., 2019). Consistent with this expectation, our analyses revealed an overall pattern of female-biased dispersal in *V. stejnegeri*, aligning with patterns previously reported in nocturnal *Protobothrops mucrosquamatus* (Yu et al., 2022), but contrasting with the male-biased dispersal observed in the island-dwelling *Gloydius shedaoensis* (Wen et al., 2021). These results unexpectedly contradicted our initial hypothesis of male-biased dispersal. However, single population-level analyses revealed variability in dispersal patterns, with the AH, JX, and YN populations exhibiting female-biased dispersal and the SC population demonstrating male-biased dispersal. This variability in sex-biased dispersal within different populations of the same species is not unusual and has been reported in other snakes. For instance, Zwahlen et al. (2021) reported male-biased dispersal in two populations of *Vipera aspis*, while no sex bias was detected in two others. Although this variation was initially attributed to habitat fragmentation, subsequent work demonstrated that habitat variation disproportionately affected males, with negligible influence on female movement (Zwahlen et al., 2021). Similarly, Lane and Shine (2011) proposed that sex-biased dispersal in sea

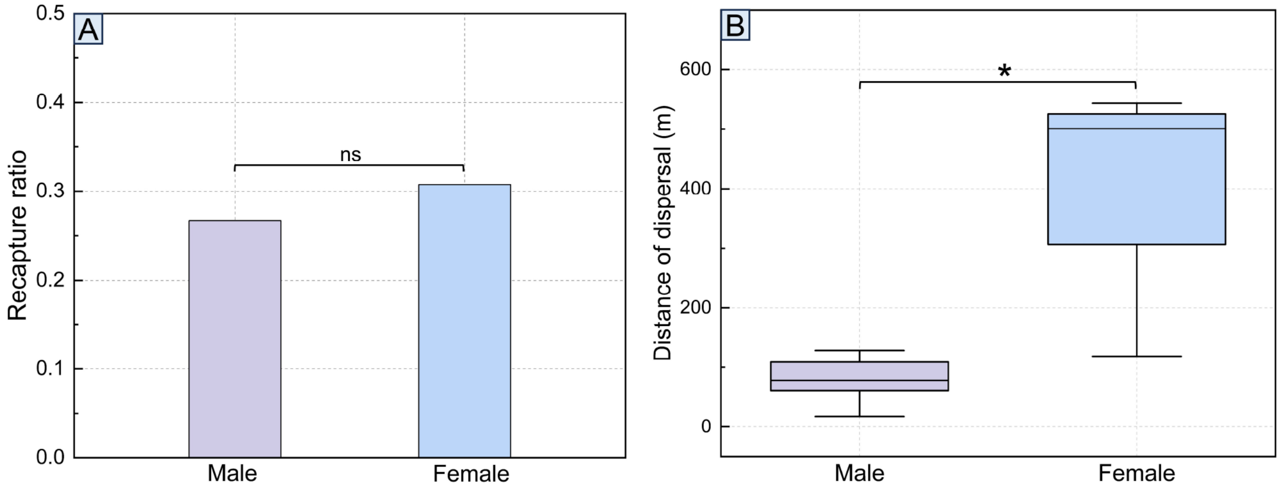

Figure 8 Mark-recapture data for the AH population of *Viridovipera stejnegeri*

A: Mark-recapture ratio post-breeding season. B: Individual dispersal distances. ns: Not significant; *: $P<0.05$.

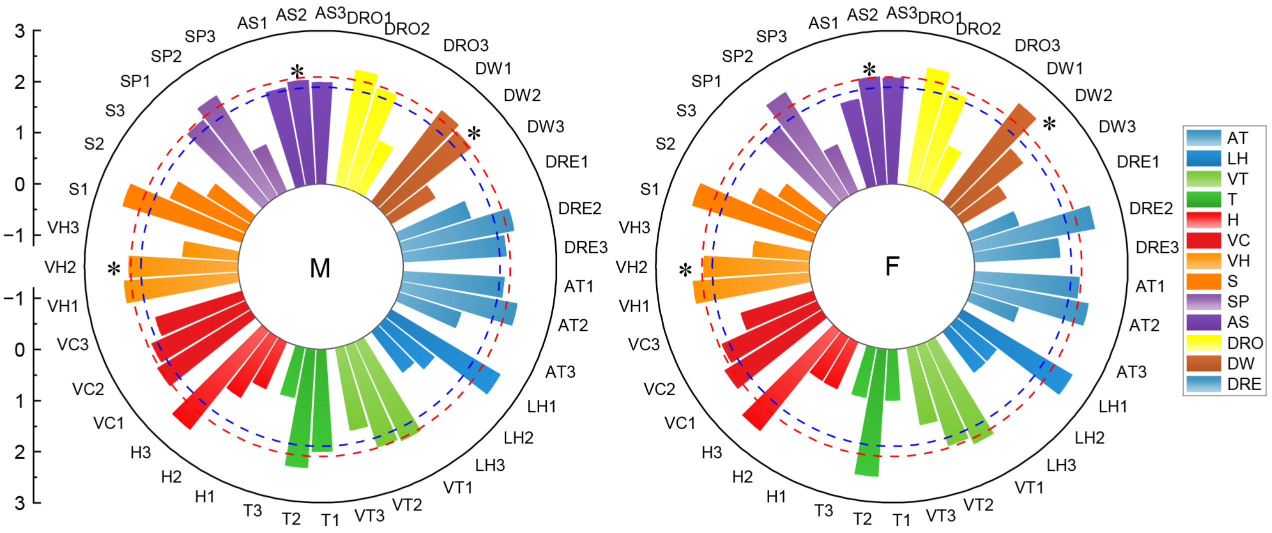

Figure 9 Habitat selection preferences of both sexes of *Viridovipera stejnegeri*

Data were plotted based on Scavia (E_i) selection index plus 2. AT: Altitude; LH: Landscape habitat; VT: Vegetation types; T: Temperature; H: Humidity; VC: Vegetation coverage; VH: Vegetation height; S: Slope; SP: Slope position; AS: Aspect slope; DRO: Distance from roads; DW: Distance from water; DRE: Distance from residential sites. Habitat factor groups 1, 2, and 3 are detailed in Supplementary Table S3. Area outside outer dashed line indicates preference, area inside inner dashed line indicates avoidance, region between two represents random selection.

snakes (*Laticauda saintgironsi* and *L. laticaudata*) may reflect differences in local food availability. In the present study, limited ecological data precluded identification of the precise environmental factors underlying population-level variation in sex-biased dispersal. Furthermore, the observed dispersal patterns did not correspond with genetic cluster boundaries, indicating that genetic structure alone is insufficient to explain these differences. These findings suggest that sex-biased dispersal in *V. stejnegeri* may be shaped more by context-dependent ecological pressures or demographic strategies than by underlying genetic differentiation (Li & Kokko, 2019).

Mark-recapture analysis of the AH population revealed no significant difference in post-breeding dispersal rates between males and females, a result that diverged from patterns inferred using microsatellite markers. However, subsequent statistical comparisons of dispersal distances aligned with the genetic evidence, indicating greater female dispersal. Discrepancies between methods used to assess sex-biased dispersal have been documented in other snakes. For instance, in the olive sea snake (*Aipysurus laevis*), male-biased dispersal was identified using mitochondrial DNA, whereas microsatellite markers showed no such bias (Lukoschek et al., 2008). In *V. stejnegeri*, the discrepancies between molecular and mark-recapture data may reflect ecological constraints such as nocturnality, arboreality, and complex terrain, which likely impeded incomplete recapture. Additional factors may include insufficient monitoring duration and the absence of juvenile data, as dispersal may occur predominantly during early life stages (Blouin-Demers & Weatherhead, 2021). These limitations underscore the challenges of relying solely on mark-recapture for ecological inference (Willson et al., 2011) and suggest that microsatellite markers may provide a more accurate assessment by avoiding incomplete sampling and inadequate monitoring duration (Dubey et al., 2008).

Moreover, spatial heterogeneity and anthropogenic landscape alteration may produce divergent dispersal dynamics across populations, necessitating attention to spatial scale in sex-biased dispersal studies (Pernetta et al., 2011; Lane & Shine, 2011). In conclusion, sex-biased dispersal in

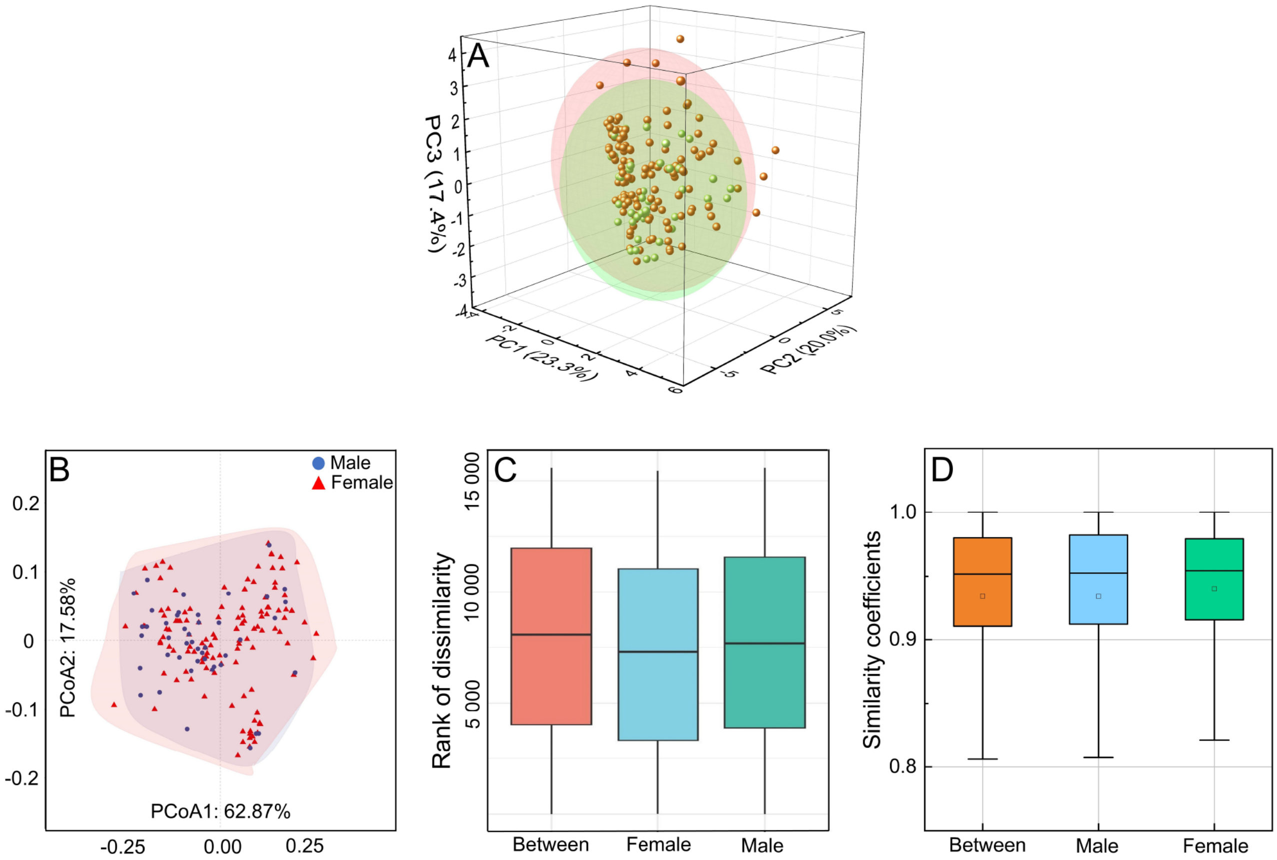

Figure 10 Multivariate comparison of habitat use between sexes in *Viridovipera stejnegeri*

A: Principal component analysis (PCA). B: ADONIS analysis. C: ANOSIM results. D: Mean similarity coefficients within and between sexes. Red and green dots represent males and females, respectively.

snakes is influenced by a confluence of ecological, demographic, and methodological factors. Future research should prioritize integrative, population-specific approaches to resolve these complexities.

Factors influencing sex-biased dispersal in *V. stejnegeri*

Sex-biased dispersal arises from the differential reproductive costs and benefits between the sexes, which shape sex-specific strategies that optimize fitness and survival (Waser & Strobeck, 1998). In birds and mammals, several hypotheses have been proposed to explain the evolutionary mechanisms behind sex-biased dispersal, among which inbreeding avoidance, local mate competition, and resource competition have gained broad support (Yu et al., 2022).

The resource competition hypothesis is frequently cited as a key evolutionary mechanism for sex-biased dispersal in snake species, such as *P. mucrosquamatus* (Yu et al., 2022). This hypothesis suggests that females—due to their higher resource demands for reproduction—tend to disperse to reduce competition for limited resources. In *V. stejnegeri*, however, habitat selection analyses revealed no significant differences between males and females across key environmental variables, suggesting that both sexes occupy similar ecological niches and experience equivalent competitive pressures. Therefore, from the perspective of habitat selection, the resource competition hypothesis cannot be substantiated. Further investigation of sex-specific resource use—such as dietary preferences, temporal activity patterns, or microhabitat partitioning—is required to assess whether sexual niche differentiation contributes to dispersal asymmetry (Tan et al., 2024). Additionally, variation in mate availability, itself a critical resource linked to mating systems, may also play a key role in shaping dispersal dynamics.

The inbreeding avoidance hypothesis proposes that one sex disperses to reduce the fitness costs associated with mating among close relatives (Vigilant et al., 2015). Although its role in sex-biased dispersal remains controversial, as kin recognition and mate selection mechanisms can effectively mitigate inbreeding without necessitating dispersal (Guillaume & Perrin, 2009; Moore & Ali, 1984), genetic evidence from *V. stejnegeri* supports this mechanism. Kinship analysis of the three AH subpopulations showed a higher proportion of consanguineous individuals between subpopulations than within them, while average relatedness did not differ significantly within or between subpopulations. IBD analysis further indicated no correlation between geographic and genetic distance. Together, these findings suggest that kin do not cluster within subpopulations but are instead redistributed to neighboring subpopulations via sex-biased dispersal, effectively reducing the risk of inbreeding. Similar patterns have been observed in other pitvipers, such as *G. shedaoensis* (Wen et al., 2021). These results support inbreeding avoidance as a plausible driver of female-biased dispersal in *V. stejnegeri*.

The local mate competition hypothesis posits that sex-biased dispersal evolves in response to asymmetries in mating competition, shaped by mating systems. In general, polygynous species tend to exhibit male-biased dispersal, as males move to maximize access to multiple females (Keogh et al., 2007). In contrast, polyandrous species are typically associated with female-biased dispersal (Rivas & Burghardt, 2005), while monogamous species exhibit similar dispersal tendencies regardless of sex (Dobson, 1982). Most snake species have traditionally been regarded as polygynous, with males seeking multiple mating partners, supporting the expectation of male-biased dispersal (Keogh et al., 2007). However, recent findings challenge this assumption, suggesting that polyandry, rather than polygyny, may be more prevalent and may reflect ancestral reproductive strategies in snakes (Rivas & Burghardt, 2005). Furthermore, the presumed link between polygyny and male-biased dispersal has been criticized as an oversimplification, lacking consistent empirical support (Li & Kokko, 2019). According to the local mate competition hypothesis, a heavily skewed sex ratio is expected to intensify male competition and reinforce female choice, thereby promoting male-biased dispersal. However, in the present study, despite a highly skewed sex ratio (4.1:1), male-biased dispersal was not detected. Currently, the mating system of *V. stejnegeri* remains poorly characterized, precluding interpretation of the observed dispersal patterns based on this hypothesis. To accurately determine the mating system and its influence on dispersal, further research is required, including field observations and kinship analyses of offspring.

In addition to mating systems, several other factors may contribute to the evolution of sex-biased dispersal, including social structure, parental investment, sexual dimorphism, and intersexual competition (Perrin & Mazalov, 2000). The emergence of multiple, non-exclusive hypotheses reflects the multifactorial and context-dependent nature of sex-biased dispersal, indicating that no single mechanism is sufficient to account for all observed patterns. Comprehensive analyses that test several competing hypotheses within the same population or species are essential for accurately elucidating the evolutionary drivers of sex-biased dispersal.

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

All animal collections obeyed the Wildlife Protection Act of China. Collection permits were issued by the Animal Care and Ethics Committee of Yibin University (YBU2020007).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

S.W.T., Q.L., and P.G. conceived the study. S.W.T., Y.Y.W., G.C.S., and B.L. conducted the field surveys. S.W.T., M.Y., and Q.L. completed the molecular experiments, analyses, and draft manuscript. S.W.T. completed the remaining analyses and prepared the manuscript, with input from other authors. All authors read and approved the final version of the manuscript.

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