Full Length Research Paper

Evaluation of pre-mating reproductive isolation in *Archachatina marginata* from three populations in the humid tropics

Ogbu, C. C* and Ugwu, S. O. C.

Department of Animal Science, University of Nigeria, Nsukka.

Three populations of *Archachatina marginata* snails (P_1, P_2 and P_3) obtained from natural snail habitats located in three states of Nigeria (one population per state) namely Enugu, Edo and River States were evaluated for pre-mating reproductive isolation using mate-choice tests. Total number of mated snails were very small (19.2%) compared to the number tested. Mating propensity (MP) varied significantly (P ≤ 0.05) among snail populations in two test groups and observed MP in the test groups differed significantly (chi-square test, P <0.05; 0.001) from that expected under random mating. Pair formation was significantly (chi-square test, P < 0.05; 0.001) influenced by differences in MP and within-population (homotypic) and between population (heterotypic) mating occurred in frequencies that differed significantly (chi-square test, P ℜ 0.05; 0.001) from that expected under random mating. Whereas observed heterotypic pair formation were less than that expected under random mating, homotypic pair formation were either equal or more than that expected under random mating. Duration of reproductive activities differed significantly (P ≤ 0.05) among test populations. It was concluded that reduced pair formation, elongated duration of courtship, and reduced mating between populations of *A. marginata*, indicate partial or incomplete pre-mating reproductive isolation.

Key words: Pre-mating isolation, mating propensity, mating duration, homotypic mating, heterotypic mating.

INTRODUCTION

Speciation is the process by which two species form from one (Grant and Grant, 2009). Among the competing species concepts, the morphological (Darwin, 1859 in Abbott et al*.,* 2008) and the biological (Mayr, 1942) species concepts are in the fore front with the biological species concept remaining the most contentious (Claridge et al*.,* 1997; Via, 2002; Coyne and Orr, 2004; Abbott et al*.,* 2008). Each of these concepts has its own strengths and weaknesses (Abbot, et al*.,* 2008; Mallet, 2008) as well as proponents and opponents (Via, 2001, 2002; Abbott et al*.,* 2008; Stadler et al., 2008). The various species concepts however, considerably agree as to what species exist in nature and about what biological forces are that explain those species. A set of organisms that interbreed and produce viable hybrids are likely to be phenotypically alike, adapt to a similar ecological niche and share a set of phenotypic characters that are used to exploit the ecological niches.

Widely separated snail populations may undergo speciation through genetic differentiation by reasons of inhabiting different geographical locations, the evolution of different character traits necessary to survive in their respective environments and reproductive isolation due to lack of contact and/or genetic, morphological, or ethological incompatibility between forms. There is presently dearth of information on the biology of *Archachatina marginata* and other members of the genus *Achatina*. *A. marginata* is the predominant and most preferred giant African land snail (GALS) in Nigeria (Raut and Barker, 2002). Among the GALS, *A. fulica* has received the greatest scientific attention probably due to its pestiferous nature (Raut and Barker, 2002; Albuquerque et al., 2008). Tomiyama (1992) studied dispersion and homing behaviour in *A. fulica* and reported a dispersion of 161 cm and 100 cm for mature and immature snails, respectively. Homing behaviour was age dependent being strong for old adults but absent in young adults and juveniles. Panja (1995) in Raut and

*Corresponding author. E-mail: coschi07@yahoo.com.

Barker (2002) reported that total distance traveled per night of activity decreased during the season irrespective of the age structure of the population with an average of 1429 cm in June reducing to 912 cm in October. Homing behaviour was 20% for animals of 40-49 mm shell size, 78% for animals of 70-79 mm shell size but absent in animals of 20-29 mm shell size (Raut and Barker, 2002). Homing in terrestrial gastropods is mediated by directional trail following and chemoreception of airborne odours from the home sites (Cook, 2001; Raut and Barker, 2002). Given the low potential for active dispersion in GALS (Takeuchi et al., 1991; Tomiyama, 1992; Albuquerque et al., 2008) and their strong homing instinct, human agents as well as formites (transport vehicles etc) have been the principal agents for the dispersion of GALS (*A. marginata, A. fulica, A. achatina*) and colonization of distant lands (Takeuchi et al., 1991; Monney, 1994; Gascoigne, 1994; Raut and Barker, 2002; Albuquerque et al., 2008). In a mating test involving species of GALS from rural Nigeria, Ugwu et al. (2011) observed the least number of mated pairs (three mated pairs out of eleven pairs tested or 23.1%) for *A. marginata*. Widely separated snail populations could hence become reproductively isolated.

Traditionally, spatial context has played a dominant role in consideration of the mode of speciation (Abbott et al., 2008). Three models: sympatric, parapatric and allopatric speciation models predominate and compete (Via, 2001, 2002; Abbott et al., 2008). Sympatric speciation is the formation of two or more descendant species from one ancestral species all occupying the same geographical location. Thus, sympatric speciation proceeds without spatial distribution and with adaptive divergence occurring under conditions of random mating (Abbott et al., 2008). Sympatric speciation is therefore, a function of differences in preferred habitats, resource allocation and utilization and the evolution of different reproductive (sexual) behaviour leading to preferred mating among genetically identical individuals possessing certain competitive advantages within a geographical location – natural (divergent) selection against the production of hybrids bringing about ecological divergence. Parapatric speciation occurs when in a geographical range gene flow is reduced because mating occurs only or more frequently among individuals that are closest. Mating is hence not random. Consequently, gene flow within the range is reduced and phenotypes gradually diverge from the extreme forms. The genetic isolation arising from the great distance separating subpopulations coupled with divergent selection eventually leads to speciation. Allopatric speciation considers the presence of geographical barriers (geographical isolation) as the predominant means by which speciation occur. The process involves the introduction of some members of a species into a new geographical location, colonization of the new location, adaptation to the peculiar characteristics/resources of the new location (adaptive divergence in

allopatry), and the development of a barrier to interbreeding when such diverged populations encounter each other in sympatry (reproductive isolation). The barrier may be formed fully in allopatry before encounter or may be partial (incomplete) or incipient at point of contact but reinforced in sympatry by natural selection that causes further divergence (reinforcement of reproductive isolation) (Coyne and Orr, 2004; Hoskin et al., 2005; Stadler et al., 2008).

The importance of geographical isolation in facilitating evolutionary divergence through mutation, genetic drift and adaptive differentiation has been recognized over the centuries, and the role of geographical isolation in speciation has become axiomatic in biology (Schneider et al., 1999). Of all the geographical speciation models, the process of allopatric speciation is the best understood and least controversial (Losos and Glor, 2003; Coyne and Orr, 2004; Stadler et al., 2008).

It is important to understand how reproductive isolation evolves because it is the final step to speciation (Weinberg et al., 1990) and the isolation barriers are of interest in understanding how biological species arise and how they are maintained (Dell'Olivo et al., 2011). Factors that bring about reproductive isolation have been classified into prezygotic (before fertilization) and postzygotic (after fertilization) isolation factors. Prezygotic isolation is the reduced probability of mating between individuals of different populations or subsets of the same population as a result of behavioural and/or phenotypic differences leading to sexual selection within and between populations (Dobzhansky, 1970; Kirkpatrick, 1982; Phelan and Baker, 1987; Grant and Grant, 2009; Dell'Olivo et al., 2011). Postzygotic isolation is the reduced viability or total inviability of hybrids formed by the mating of individuals from reproductively isolated populations. It is postulated that genes that have diverged in allopatric populations become incompatible when combined in hybrids causing hybrid sterility or inviability (Abbott et al., 2008; Dell'Olivo et al., 2011).

To test whether allopatric (geographically isolated) populations of *A. marginata* constitute different organisms (different species) would require the demonstration of reproductive isolation among individuals of those populations in sympatry (Abbott et al., 2008). Mate choice tests investigating courtship duration, mate selection (mate preferences), and duration of mating (DM) among adults of such populations could reveal the presence of total, partial or incipient premating reproductive isolation. We hereby report an experiment designed to determine the presence of premating reproductive isolation in *A. marginata* obtained from three states in humid tropical Nigeria.

MATERIALS AND METHODS

The study was carried out in the snailery unit of the department of

Table 1. Test population, courting pairs and mated number of snails for the mate test experiment.

Test population	Test unit (Tetrad number)	Courting snail (pair)	Mated snail (pair)	Mated snail as % of courted snail	Mated snail as % of tested snail
P_1 x P_2	18	9	5	55.6	13.9
P_1 x P_3	19	11	9	81.8	23.7
P_2 x P_3	15	7	6	85.7	20.0

animal science, university of Nigeria, Nsukka from December, 2007 to October, 2008. A total of 208 adult *A. marginata* snails (mean size = 12 cm) of unknown reproductive and evolutionary history were randomly collected from three populations (P_1, P_2 and P_3) located in three states (one population per state) namely Nsukka in Enugu State (eastern Nigeria), Warri in Delta State (south eastern Nigeria) and Port-Harcourt in River State (south-south Nigeria) for P_1, P_2, and P_3, respectively. At each sampling location, snails within an area not exceeding 20 x 20 m were sampled based on the dispersive ability of the snail species. On arrival, the snails were washed by spraying, marked and isolated in individual plastic baskets measuring 16 cm in diameter by 10 cm high. The bottom of each basket was covered by 3 cm thick garden soil. The snails were fed a combination of formulated ration and plant food materials. Isolation in individual baskets lasted for at least 90 days (range 90 days to 120 days) believed to be long enough to allow the snails develop strong desire to mate (Dillen et al., 2010) and to shed fertilized eggs it may have carried from the wild (Baur, 1993; Landolfa et al., 2001).

Mating arrangement

At the end of the isolation period, two snails were randomly selected from one population and housed together with that from another population in a plastic basket measuring 60 x 45 x 20 cm to form a test unit (a tetrad). The containers were wide enough to allow free movement of the snails within the test area (Baur and Baur, 1992). Between 15 and 19 replications of each tetrad were set up giving 52 test units (tetrads) for three population combinations (P_1 x P_2; P_1 x P_3 and P_2 x P_3).

Parameters measured

The snails in each test unit were observed from 6.00 pm to 6.30 am daily for courtship latency (CL) (time from initial association or meeting to initiation of oral contact), duration of courtship (DC) (time from initiation of oral contact to onset of copulation), and duration of copulation or mating (time from onset of copulation to separation of the snails). Each test unit was observed initially for 12 h (6.00 pm to 6.00 am) and if no snail initiated courtship, observation was terminated. Observation was also terminated for each test unit as soon as the first copulation occurred in a test arena since the remaining two snails will have no mate choice (Baur and Baur, 1992; Baur, 1993). Each snail participated once in the mate test. Association (courtship and/or mating) between two snails from the same population was termed homotypic courtship or mating while that between snails from different populations was denoted as heterotypic courtship or mating. The mating propensity (MP) of snails of each population was obtained as the percentage of snails of that population that mated in each test group. The snailery is an enclosure made of expanded metal and chick wire mesh with open roof and sides. Creeping plants covered the roof of the enclosure and provided shading from the sun. Shaded kerosene lantern in addition to moon light provided enough illumination for observation without disturbing the nocturnal habit of the snails.

Experimental design and data analysis

The experimental design was a completely randomized design (CRD) in one-way classification. Data on duration of courtship latency, courtship and mating were analyzed using the analysis of variance (ANOVA) option of SPSS statistical package (SPSS, 2001). Significant means were separated using the Duncan option of SPSS. To determine the effect of different mating propensities on pair formation, the expected frequencies of pair formation under random mating and after correcting for differences in MP were calculated for each test population and compared with observed frequencies using the chi-square test for goodness of fit. The mating propensities of the two snail populations that make up a test population as well as that of the same snail population in different test groups were also compared using the chi-square (x^2) test for goodness of fit.

RESULTS AND DISCUSSION

Table 1 presents the number of tested units (groups of four snails or tetrads), courted snails (pairs), mated snails (pairs) and percentages of tested and courted snails that mated. The table showed that out of 36 pairs of snails (eighteen tetrads) tested for P_1 x P_2 population, only nine pairs (25%) established courtship while only five pairs (13.9% of tested pairs or 55.6% of observed courting pairs) succeeded in mating. Across test populations percentage of courted snails and tested snails that mated ranged from 55.6-85.7% and 13.9-23.7%, respectively. The overall mean percentage of established courtships and courtships that led to mating was 26% and 19.2% of tested pairs, respectively indicating that over 70% of the tested pairs of snails did not court at all or broke courtship within CL while over 80% either did not court at all or courted but did not mate.

The wide differences between number of tested snails and the number that eventually mated in each test group indicate that there was high mate selectivity among the snails tested. However, most pairings that successfully courted also succeeded in mating (55.6%, 81.8%, and 85.7% for P_1 x P_2, P_1 x P_3, and P_2 x P_3 test groups, respectively (mean 74.1%). The mean value of 19.2% (range, 13.9 – 23.7%) reported in the present study as percentage of tested snails that mated is comparatively higher than the 10% reported by Tomiyama (1994) in an experiment that studied reproductive behavior of *A. fulica* under field situation. The snails used in the present study

Table 2. Mating type, number of mating pairs, number of mated snail type, percentage of mated type and MP of snails from different populations.

Test population	Mating (type)	Pair (number)	Mated snail (type) (number)		Mated type (%)		MP (%)	
			P_i	P_j	(Homo-)	(Hetero-)	(P_i)	(P_j)
$P_1 \times P_2$	$P_1 \times P_1$	1						
	$P_1 \times P_2$	2	4	6	60.0	40.0	11.11	16.67
	$P_2 \times P_2$	2						
$P_1 \times P_3$	$P_1 \times P_1$	2						
	$P_1 \times P_3$	3	7	11	66.7[a]	33.3[b]	18.42[a]	28.95[b]
	$P_3 \times P_3$	4						
$P_2 \times P_3$	$P_2 \times P_2$	1						
	$P_2 \times P_3$	1	3	9	83.3[a]	16.7[b]	10.00[a]	30.00[b]
	$P_3 \times P_3$	4						

Means on the same row with different superscripts are significantly different; $P \leq 0.05$.

were collected from the wild during the dry season in Nigeria (December to February) when snails go into aestivation and were kept individually until tested for mating within the rainy months (from March) when snails usually resume active reproductive activities. Snails have been shown to have high propensity to mate after arousal from dormancy (Baur and Baur, 1992; Baur, 1993) and/or after long period of isolation (Dillon et al., 2007; Dillen et al., 2010). In a study that considered only courtships that led to mating in *Arianta arbustorum*, Baur and Baur (1992) reported the percentage of snails that successfully courted (and therefore mated) as 20.4% indicating that over 70% of tested snails either did not court at all or courted unsuccessfully which compared favourably with our findings.

Table 2 presents the type and number of mating pairs, number of each snail type mated, percentage of each type of mating, and mating propensities of snail types (snails from different populations). The table showed that among mated types, homotypic mating was more frequent (range, 60.0%-83.3%) than heterotypic mating (range, 16.7%-40.0%) within each test population. The lower proportion of heterotypic pairs that eventually mated across test populations compared to homotypic pairs suggest that there was very low number of heterotypic pairing for courtship or that most heterotypic pairings did not successfully court or mate. In the experiment with *A. arbustorum* (Baur and Baur, 1992), courtship was more frequently broken off in heterotypic pairs (in ten out of twenty-three cases; 43.5%) than in homotypic pairs (in three out of thirty-nine cases; 7.75%).

The higher frequencies of homotypic mating compared to heterotypic mating within and across test populations could be attributed to greater compatibility between snails of the same population than between those from different populations. Shared mating experience, similar or familiar reproductive behaviour, sex signaling, sensory, hormonal and tactile cues are all important pre-mating factors which influence both the rate and volume of reproductive activities in snail populations (Phelan and Baker, 1987; Zeeck et al., 1988; Frey et al., 1998; Hankison and Morris, 2003; Jang et al., 2009; Koene, 2010). Olfaction and tactile cues are known to play decisive roles in mate selection during courtship and mating in snails (Horth, 2007; Tekeichi et al., 2007). Sex pheromones secreted by opposite sexes or potential snail mates must be identified and recognized as such before successful courtship can be established. Lind (1976) reported that reproductive behaviour in the land snail, *Helix pomatia*, is highly organized and sequential, and can be stereotyped. Recognition of these sexual signals requires experience hence snails that have shared the same environment and probably interacted sexually in the past are more likely to recognize, respond to and reciprocate mating cues released or exhibited by members of the same population than heterotypes. Since mating is completed only when there is appropriate exchange of stimuli, mate preference was more for homotypes than for heterotypes. Reproduction is a costly activity in snails (Daly, 1978; Ridly, 1983; Landolfa et al., 2001; Saltin, 2010) hence snails invest reproductive resources to maximize reproductive fitness (Landolfa et al., 2001; Johannesson et al., 2010). Accordingly, homotypic pairing in the present experiment seems natural, sure and more beneficial hence optimal while hetrotypic pairing could be regarded as suboptimal and maladaptive (Saltin, 2010). Reports on the mate preferences of *A. marginata* snails from different populations are almost non existent in literature however, collaborative studies involving other species report similar between population mate pre-ferences. For instance, in the experiment with *A. arbustorum* (Baur and Baur, 1992), the number of homo and heterotypic matings in their three test populations were 9 vs 4; 19 vs 3 and 8 vs 6, respectively indicating

that homotypic mating accounted for 69, 86 and 57%, respectively (mean 70.7%) of all matings in the three test populations which are in high accord with our values of 60%, 66.7%, and 83.3% (mean 70%) obtained in the present study. Sutton et al. (2005) using mate selection tests studied chemical signal mediated premating reproductive isolation in the river worm, *Neanthes acuminata* and reported that all populations were found to preferably mate with members of their own population. In this experiment, heterotypic pairs showed aggression even in cases where the distance between two of the populations studied was less than 15 miles.

The reduced mating between heterotypes indicate the presence of pre-mating reproductive isolation between *A. marginata* snails separated by distance and inhabiting different geographical locations while non-mating homotypes signal pre-mating reproductive isolation probably due to sexual selection on account of character displacement (ethological barrier) (Amanda et al., 2004) or different levels of interaction (ecological interaction) between the snails and their environment (Via, 2002). Isolating mechanisms preventing populations of the same animal species from coming in contact include geographical isolation (allopatric distribution), isolation due to distance (like in animals with limited locomotive ability), climate and seasonal barriers. These mechanisms prevent migration and intermingling of the individuals of the two populations, reducing or preventing exchange of genes between them so that the populations are genetically out of contact with each other and follow an independent evolutionary path. Consequently, both populations genetically differentiate and diverge, become distinct and form separate units. Some other isolation mechanisms act within as well as between populations. Sexual selection reproductively isolate members of the same (sympatric) population as well as members of different (allopatric) populations held in sympatry. Premating reproductive isolation by these mechanisms are usually due to differences in courtship behaviour displayed by different animals (Moehring et al., 2004). Species recognition or mate recognition usually is a consequence of the display of familiar courtship behaviour. Males show specific mating behaviours and females must respond positively to these displays before mating can occur. Changes in courtship behaviour or non recognition of courtship behaviour (leading to mate rejection) can be a sign of incipient premating reproductive isolation (Dobzhansky, 1970; Weinberg et al., 1990; Baur and Baur, 1992; Tregenza et al., 2000; Via, 2002; Grant and Grant, 2009; Schluter and Conte, 2009; Abbott et al., 2009).

Table 2 also shows that MP varied significantly (p≤0.05) between snails in two out of the three test populations. For instance in test group P_1 x P_3, P_3 snails significantly (chi-square test; P ≤ 0.05) surpassed P_1 snails in MP (28.95% vs 18.42%) accounting for 61.1% of mated snails as against 38.9% for P_1 snails. P_3 x P_3

homotypic mating was correspondingly higher in frequency than P_1 x P_1 homotypic mating (four out of nine pairs or 44.4%). In P_2 x P_3 test group, the picture was quite similar with P_3 snails being highly significantly (chi-square test; p≤0.001) superior in MP to P_2 snails (30.00% vs 10.00%) equivalent to 75% and 25% of mated snails, respectively. P_3 x P_3 homotypic mating was thus higher than P_2 x P_2 homotypic mating in frequency (66.7% vs 16.7%). The higher MPs observed for non-resident snails (P_3 snails) over resident type (P_1 snails) in P_1 x P_3 test population was quite interesting. We could not find any plausible reason for this observation since the snail samples used in this study were collected from natural populations exposed to similar climatic conditions (the three populations sampled belonged to the rain forest, humid ecological zone of Nigeria). Although, Nsukka the site of this experiment and source of P_1 snails is on the average cooler (mean daily temperature, 25°C) than Sapele and Port Harcourt (sources of P_2 and P_3 snails, respectively), all the snails were allowed at least 90 days to adapt to the experimental environmental conditions. Other mate test experiments (Baur and Baur, 1992; Fearnlay, 1995; Baur et al., 2009) reported similar significant differences in MP between resident and immigrant snail populations that could not be attributed to any obvious reasons. Baur and Baur (1992) however, suggested that snails from more benign conditions exhibit significantly lower MP which is in accord with our findings with respect to P_1 snails but could not account for the very low MP observed for P_2 snails in P_2 x P_3 test population. Perhaps, it may be that intra-population (homotypic) attraction or compatibility differed from one population to another so that more homotypic pairing occurred in favour of homotypes with stronger attraction or greater compatibility. However, inter population differences in MP have often been considered as the first step to premating reproductive isolation (Meffert and Bryant, 1991; Baur and Baur, 1992; Fearnley, 1995; Tregenza et al., 2000; Via, 2002; Grant and Grant, 2009; Schluter and Conte, 2009; Abbott et al., 2009).

Table 3 compares observed to expect MP of snails from different populations under random mating as well as the MP of the same snails in different test populations. The chi-square test for goodness-of-fit revealed that observed MP deviated significantly (chi-square test; P < 0.05; 0.001) from that expected under random mating in P_1 x P_3 and P_2 x P_3 test groups. MP values for P_1 snails were similar in different test populations (chi-square test; P ≥ 0.1) but differed significantly for P_2 and P_3 snails (chi-square test; P < 0.05; 0.001) in different test groups. MP (readiness or desire to mate) is both genetic and environmental and will usually differ within and between populations. The more active a snail is, the more readily it will initiate courtship (Baur and Baur, 1992). Significant variation in MP due to snail combination could arise from differences in strength of attraction between homotypes and/or differences in compatibility between heterotypes

Table 3. Chi-square test for MP of different snail populations in different test groups.

Test population	Trials (number)	Snail population	Mating propensity		Chi-square statistic (χ^2)[*1]		P
			(observed)	(expected)	(calculated)	(tabulated)	
$P_1 \times P_2$	18	P_1	11.11	11.56	2.28	2.71	$\wp$ 0.10
		P_2	16.67	11.56			
$P_1 \times P_3$	19	P_1	18.42	19.74	4.39	3.84	$\Re$ 0.05
		P_3	28.95	19.74			
$P_2 \times P_3$	15	P_2	10.00	16.67	13.32	7.88	$\Re$ 0.001
		P_3	30.00	16.67			
$P_1 \times P_2$; $P_1 \times P_3$		P_{12}	11.11	11.56	0.11	2.71	$\wp$ 0.10
		P_{13}	18.42	19.74			
$P_1 \times P_2$; $P_2 \times P_3$		P_{21}	16.67	11.56	4.93	3.84	$\Re$ 0.05
		P_{23}	10.00	16.67			
$P_1 \times P_3$; $P_2 \times P_3$		P_{31}	28.95	19.74	14.96	7.88	$\Re$0.001
		P_{32}	30.00	16.67			

P_{12}, P_1 snails in $P_1 \times P_2$ test population; **P_{13}**, P_1 snails in $P_1 \times P_3$ test population; **P_{21}**, P_2 snails in $P_1 \times P_2$ test population; **P_{23}**, P_2 snails in $P_2 \times P_3$ test population; **P_{31}**, P_3 snails in $P_1 \times P_3$ test group; **P_{32}**, P_3 snails in $P_2 \times P_3$ test group; [*1] **d.f**, 1 in all cases.

all of which influence mate formation. The significantly (P < 0.05) higher MP observed for P_3 snails against P_1 and P_2 snails in $P_1 \times P_3$ and $P_2 \times P_3$ test groups, correlated with the significantly higher mating frequencies observed for P_3 snails in these populations.

Table 4 presents the observed and expected frequencies of pair formation under random mating and after correcting for differences in mating propensities. The chi-square test for goodness-of-fit of observed mating frequencies to that expected under random mating shows that observed pair formation differed from that expected under random mating in $P_1 \times P_3$ and $P_2 \times P_3$ test groups (chi-square test; P < 0.05; 0.001). In all test populations, there were more homotypic matings than expected while fewer heterotypic matings were observed. The variation between observed and expected frequencies of pair formation indicate the influence of forces of sexual selection operating through differences in attraction, mating propensities and/or compatibilities between snails of different populations to influence pair formation (Baur and Baur, 1992). The observed higher frequencies of homotypes further confirm the greater attraction and/or compatibility between snails from the same population compared to that for different populations while the lesser frequencies of heterotypes reinforce our inference of the presence of premating reproductive isolation in this species. Table 4 also shows that differences between observed and expected frequencies of pair formation reduced in significance (from P < 0.001 to P < 0.05) for $P_2 \times P_3$ test group and became insignificant for $P_1 \times P_3$

test population after correcting for differences in MP confirming that differences in MP significantly influenced pair formation in these test groups.

Figure 1a, b, and c compares the reproductive parameters (duration of CL, courtship and mating) for mated types in the various test populations. CL differed significantly (P ≤ 0.05) among mating types in $P_1 \times P_2$ and $P_1 \times P_3$ test populations (Figure 1a and 1b, respectively) but was similar for mating types in $P_2 \times P_3$ test group (Figure 1c). For DC and DM, significant differences (P ≤ 0.05) were obtained among mating types in $P_1 \times P_3$ and $P_2 \times P_3$ test groups (Figure 1b and 1c, respectively). Whereas heterotypic mating had overall lower mean CL across populations compared to homotypes (93.5 min vs 114.7 min), homotypic mating had overall lower mean DC (199.2 min vs 251.8 min) but higher overall mean DM (249.1 min vs 188.9 min) compared to heterotypes. The shorter duration of CL for heterotypes indicate that non-compatible or non-accepting heterotypes broke initial (tentacular) contact faster than non-accepting homotypes. Baur and Baur (1992) reported that incompatible hetero- and homotypes in *A. arbustorum* broke initial contact within mean time of 5.5 ± 0.6 min (range 1 to 15 min) or courtship began with oral contact. In the same report, CL ranged from 81.6 ± 41.0 min to 221.2 ± 10.1 min (mean 129.2 min) for heterotypes and 27.0 min to 229.9 ± 59.2 min (mean 166.6 min) for homotypes indicating that CL varied widely between courting types. The higher mean DC and lower mean DM for heterotypes indicate that these groups invested more time (and

Table 4. Observed and expected frequencies of pair formation under random mating and different MPs.

Test population	Number of mating	Mating type	Mated (number)	Expected frequency (random mating[*1])	χ^{2*2}	P	Expected frequency (corrected for MP)	χ^{2*2}	P
P$_1$ x P$_2$	5	P$_1$ x P$_1$	1	0.83			0.5		
		P$_1$ x P$_2$	2	3.33	2.22	NS	3.2	1.32	NS
		P$_2$ x P$_2$	2	0.83			1.3		
P$_1$ x P$_3$	9	P$_1$ x P$_1$	2	1.5			0.9		
		P$_1$ x P$_3$	3	6	5.83	ℜ0.05	5.8	3.76	NS
		P$_3$ x P$_3$	4	1.5			2.4		
P$_2$ x P$_3$	6	P$_2$ x P$_2$	1	1			0.2		
		P$_2$ x P$_3$	1	4	11.25	ℜ0.001	3.1	5.25	□0.05
		P$_3$ x P$_3$	4	1			2.7		

[*1], Expected frequency under random mating do not take into account differences in mating propensity; [*2] d.f, 1 in all cases; N.S, not significant; χ^2, chi-square statistic; P, significant level.X.
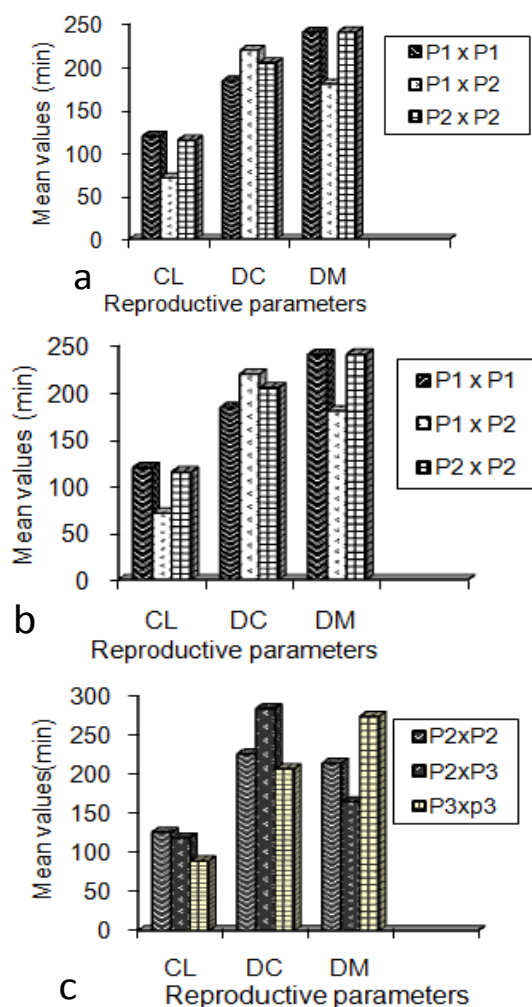

Figure 1. (a) Comparison of reproductive parameters for P1xP2 test population; **(b)** comparison of reproductive parameters for P1xP3 test population; **(c)** comparison of reproductive parameters for P2XP3 test population.

energy) in courtship but lesser time in copulation than homotypes. The longer DC was probably as a result of behavioural incompatibilities that needed to be overcome while the shorter DM could be a strategy for time management (a trade off) given that the complete sexual activities from courtship to mating must occur within the nocturnal period. Homotypes on the other hand had shorter DC which could be as a result of greater ethological (sexual and behavioural) similarity and synchrony consequent upon previously shared reproductive experiences (Hankison and Morris, 2003; Jang et al., 2009) thus saving time for longer DM. Time management is probably very critical for safety and successful reproduction in *A. marginata*. Baur and Baur (1992) reported comparable mean DC of 233.1 min (range 120.0 min to 309.9 ± 66.4 min) for homotypes and 237.4 min (range 197.6 ± 33.1 min to 273.0 ± 43.0 min) for heterotypes in *A. arbustorum* from five populations. The reported values in the present work and the range of published values from other studies (Baur and Baur, 1992; Tomiyama, 1994; Raut and Barker, 2002) indicate that DC varies widely among snail species and between individuals within the same species. Raut and Barker (2002) reported that DC in *A. fulica* lasts between 6-8 h (360 min to 480 min) but can vary from 1 h (60 min) to 24 h (1440 min). Tomiyama (1994) observed DC of less than 5 min in *A. fulica* under field observation while Chase (2007) reported that courtship in *A. fulica* can last up to 30 min (½ h).

Different species evolve different mechanisms to moderate the duration of reproductive activities. For instance, in *H. pomatia*, dart shooting is a signal to end courtship and initiate copulation (Lind, 1976). Tomiyama (1994) reported average copulation time of 4.6 h (276 min; range 1.5-7.5 h or 90-450 min) for *A. fulica* under natural (field) mating. In a mate choice experiment involving two species of *Littorina* snails, Saltin (2010)

found that male *L. fabalis* followed female *L. obtusata* for longer time than conspecific females but had longer duration of mating with conspecific females than with interspecific females. There is dearth of data on duration of mating for *A. marginata*. The range of values we obtained in the present study as well as those reported for *A. fulica* indicate that DM like other reproductive behaviors varies considerably within and across species, populations and environments. Generally, copulation in terrestrial snails is known to last from a few minutes to several hours (Plummer, 1975; Tomiyama, 1994).

Conclusion

In general, the results obtained in the present study indicated partial premating (prezygotic) reproductive isolation in *A. marginata* snails. Mate discrimination between heterotypes was most serious between P_3 snails from Port-Harcourt (River State) and P_2 snails from Warri (Delta State) with the least number of heterotypic mating. P_1 snails from Nsukka (Enugu State) were highly compatible with P_2 and P_3 snails for reasons we do not yet understand. Consequently, most of the heterotypic matings involved P_1 snails in the test populations. The mechanism of mate discrimination in *A. marginata* is yet to be studied. However, the very low number of mated snails compared to number tested suggest, that intra- and inter population mate selection and discrimination exist. It has been shown that previous (earlier) mating experience can influence mate choice (O'Hara et al., 1976; Baur and Baur, 1992). The snails used in the present study were collected as adults from their natural habitats. It is hence safe to assume homotoypic mating experience from previous years. This shared reproductive experience was posited as being responsible for the higher frequency of homotypic mating compared to heterotypic mating.

Interpretation of mate test experiments is usually handled with caution (Baur and Baur, 1992; Baur et al., 2009). This is because of the multiplicity of environmental adaptations and genetic factors including conflicting sexual interests and preferable traits that influence and confound observations on life history patterns in gastropods (Hankison and Morris, 2003; Hollander et al., 2005; Baur et al., 2009). Explaining and drawing conclusions on interpopulation differences in life-history patterns require an understanding of the influence of snail origin, snail habitat, substratum type and local climate as well as specific genetic adaptations (co-adaptations) to peculiar environmental conditions (Baur et al., 2009). Despite these limitations comparative studies on life history patterns (courtship interaction, mate selection and mating propensities) of geographically isolated popu-lations are useful in understanding the basis for the variation in reproductive behaviour of not only populations of different species, but also different populations of the same species and such studies are prerequisites to experimental studies of reproductive isolation between populations (Baur and Baur, 1992; Frey et al., 1998; Johannesson et al., 2010).

Based on the observed reduced mating between *A. marginata* snails from different populations, the elongated duration of courtship and reduced MP in some populations we conclude that partial or incomplete pre-mating reproductive isolation exist between these snail populations.

REFERENCES

Abbott RJ, Ritchie MG, Hollingswortt PM (2008). Introduction. Speciation in plants and animals: pattern and process. Phil. Trans. R. Soc. B. (363): 2965 – 2969.

Albuquerque FS, Peso-Aguiar MC, Assuncao-Albuquerque MJT (2008). Distribution, feeding behaviour and control strategies of the exotic land snail Achatina fulica (Gasptropoda: Pulmonata) in the Northeast of Brazil. Braz. J. Biol. 68(4): 837 – 842.

Amanda JM, Jian L, Malcolm DS, Shelly GS, Mathew DA, Trudy FCM, Jerry AC (2004). Quantitative trait loci for sexual isolation between *Drosophila simulans* and *D. mauritiana*. Genetics, 167: 1265 – 1274.

Baur A (1993). Experiment to test whether different forms of the land snail *Arianta arbustorum* (*Limaeus,* 1758) (pulmonata: Helicidae) are reproductively isolated. Ann. Mus. Wien 94(95): 323-333.

Baur A, Minoretti N, Baur B (2009). Effect of soil type and adult size on mating propensity and reproductive output in two populations of the land snail Arianta arbustorum (Linnaeus). Malacologia, 51(1): 1 – 11.

Baur B, Baur A (1992). Reduced reproductive compatibility in Arianta arbustorum (Gastropoda) from distant populations. Heredity. 69(1992): 65–72.

Chase R (2007). Gastropod reproductive behaviour. Scholarpedia, 2(9): 4125. available online at doi:10.4249/scholarpedia. 4125.

Clatridge MF, Dawah HA, Wilson MR (1997). Specie: the unit of biodiversity. London, UK: Chapman Hall.

Cook A (2001). Behavioural ecology: on doing the right thing, in the right place at the right time. In. Baker GM (ed.). The Biology of Terrestial Moluscs. CAB. Inter. Wallingford. pp. 447-487.

Coyne JA, Orr HA (2004). Speciation. Sinauer and Associates, Sunderland, Massachusetts.

Daly M (1978). The cost of mating. American Naturalist, 112: 771–774.

DellOlivo A, Hoballah ME, Gubitz T, Kuhlemeier C (2011). Isolation barriers between Petunia axillaries and Petunia integrifolia Solanaceae. Evolution, 65(7): 1979 – 1991.

Dillen L, Jordaens K, Van Dongen S, Backeijau T (2010). Effect of body size on courtship role, mating frequency and sperm transfer in the land snail succinea putris. Anim. Behav. 79(5): 1125 –1133.

Dillon.Jr. RT, Robinson JD, Wethington RA (2007). Empirical estimates of reproductive isolation among the freshwater pulmonate snails physa acuta, *P. pomilia*. *P. hendersoni*. Malacologia, 49(2): 283 – 292.

Dobzhanski T (1970). Genetics of the Evolutionary process. Columbia University Press, New York.

Fearnley R (1995). Heterogenic copulatory behaviour produces non random mating in laboratory trials in the land snail Helix aspersa muller. J. Mollus. Stud. 62(2): 159 –164.

Frey MA, Lonsdale DJ, Snell TW (1998). The influence of contact chemical signals on mate recognition in harpacticoid copepod. Phil. Trans. R. Soc. London. 353: 745 – 751.

Gascoigne A (1994). The biogeography of land snails in the Islands of Gulf of Guinea. Biodiv. Conserv. 3: 794 –807.

Grant PR, Grant RB (2009). The secondary contact phase of allopatric speciation in Darwin's finches. Proceed. Nat. Acad. Sci. 106(48): 20141–20148.

Hankison SJ, Morris MR (2003). Avoiding a compromise between sexual selection and species recognition: female Swordtail fish asses multiple species specific cues. Behav. Ecol. 14: 282 – 287.

Hollander J, Lindegarth M, Johannesson K (2005). Local adaptation but not geographical separation promotes assortation mating in snail. Anim. Behav. 70: 1209–1219.

Horth L (2007). Sensory genes and mate choice: Evidence that duplications, mutations, and adaptive evolution alter variation in mating cue genes and their receptors. Genomics, 90: 159–175.

Hoskin CJ, Haggie M, McDonald KR, Moritz C (2005). reinforcement drives rapid allopatric speciation. Nature, 437: 1353–1356.

Jang Y, Won YJ, Choe JC (2009). Convergent and divergent patterns of morphological differentiation provide more evidence for reproductive character displacement in a wood Cricket Gryllus fultoni (Orthoptera: Gryllidae). BMC Evol. Biol. 9: p. 27.

Johannesson K, Saltin SH, Duranovic I, Havenhand JN, Jonsson PK (2010). Indiscriminate males: mating Behaviour of a marine snail compromised by a sexual conflict? PLOS ONE 5(8): el2005. doi:10.1371/ j.pone.0012005.

Kirkpatric M (1982). Sexual Selection and Evolution of Female Choice. Evolution, 36: 1–12.

Koene JM (2010). Neuro endocrine control of reproduction in hermaphroditic freshwater snails: mechanisms and evolution. Frontiers. Behav. Neuro Sci. 4(167): 1–17.

Landolfa MA, Green DM, Chase R (2001). Dart shorting influences paternal reproductive success in the snail Helix aspersa (Pulmonata, stylommatophora). Behav. Ecol. 12(6): 773–777.

Lind H (1976). Causal and functional organization of the mating behaviour sequence in Helix pomatia Pulmo. Gastro. Behav. 59(314): 162–202.

Losos JB, Glor RE (2003). Phylogenetic comparative methods and the geography of speciation. Trends Ecol. 18: 220–227.

Mallet J (2008). Hybridization, ecological races, and the nature of species: empirical evidence for the case of speciation. Phil. Trans. R. Soc. B. 363: 2971-2986.

Mayr E (1942). Systematics and the origin of species. New York, NY: Columbian University Press.

Meffert LM, Bryant EH (1991). Mating propensity and courtship behaviour in serially bottlenecked lines of the housefly. Evolution, 45: 293–306.

Monney KA (1994). Notable notes on gaint African land snails. Snail Farming Res. 5: 1–3.

O'Hara E, Pruzan A, Ehrman L (1976). Ethological isolation and mating experience in Drosophila paulistorum. Proc. Nat. Acad. Sci. USA. 73: 975–976.

Phelan RL, Baker TC (1987). Evolution of male pheromones in moths: reproductive isolation through sexual selection? Sci. NY. 235: 205–207.

Plummer JM (1975). Observation on the reproduction, growth longevity of a laboratory colouy of Archachatina (Ealachatina) marginata (Swauison). Proc. Malac. Soc. Lond. 175(41): 395–413.

Raut SK, Barker GM (2002). Achatina fulica Bowdich and other Achatinidae as pests in tropical agriculture; In: Molluscs as crop pests. Barker GM. CAB Int. (ed.). pp. 55–114.

Ridley M (1983). The explanation of organic diversity. The comparative method and adaptations for mating. Clarendon press, Oxford, UK.

Saltin SH (2010). Complex male mate choice in marine snails Littorina. Licentiate thesis. Dept. Marine Ecol. Univ. Gothenburg.

Schluter D, Conte GL (2009). Genetics and ecological speciation. Proceed. Nat. Acad. Sci. 106(1): 9955–9962.

Schneider CJ, Smith TB, Larison B, Moritz C (1999). A test of alternative models of diversification in tropical rainforests: ecological gradients vs. rainforest refugia. PNAS, 96(24): 13869–13873.

SPSS (2001). Statistical Package for social sciences SPSS Inc. Chicago USA. 13: 0.

Stadler T, Arunyawat U, Stephen W (2008). Population genetics of speciation in two closely related wild tomatoes (Solanum section Lycopersicon). Genetcs, 178: 339-350.

Sutton R, Bolton E, Bartels-hardege HD, Eswards M, Reish DJ, Hardege JD (2005). Chemical signal mediated premating reproductive isolation in a marine pohychaete, Naanthes acuminate (arenaceodentata) J. Chem. Ecol. 31(8): 1865–1876.

Ugwu SOC, Ogbu cc, ikechuno IK (2011). Reproductive characterization of Giant African Land snails (Gals) Afr. J. Biotechnol. 10 (50): 10315-10319.

Takeuchi K, Koyano S, Numazawa K (1991). Occurrence of the Giant African Snail in Ogasawara Islands Japan. Micronesica, 3: 109–116.

Tekeichi M, Hirai Y, Yusa Y (2007). A waterborne sex pheromone and trail following in apple snails, Pomacea canaliculata. J. Mollus. Stud. 73(3): 275–278.

Tomiyama K (1992). Homing behaviour of the gaint African snail, Achatina fulica Ferussac (Gastropoda: Pulmonata). J. Ethol. 10(2): 139–147.

Tomiyama K (1994). Courtship behaviour of the Giant African snail, Achatina fulica (ferussac) (stylommatophora: achatinidae) in the field. J. Molluscan Stud. 60: 47-54.

Tregenza T, Pritchard VL, Butlin RK (2000). The origins of premating reproductive isolation: Testing hypothesis in the grasshopper Chorthippus parallelus. Evolution, 54(5): 1687–1698.

Via S (2001). Sympatric speciation in animals: the ugly duckling grows up. Trend Ecol. Evol. 16(7): 381-390.

Via S (2002). The Ecological Genetics of speciation. Arnevican Naturalist. 159: 51-57.

Weinberg JR, Starczak VR, Mueller C, Pesch GC, Lindsay SM (1990). Divergence between populations of a monogamous polychaete with male parental care Premating isolation and chromosome variation. Marine Biol. 107: 205-213.

Zeeck E, Hardege J, Bartelels-Hardege H, Wesselmann G (1988). Sex pheromone in a marine polychaete. determination of the chemical structure. J. Exp. Zool. 246: 285–292.