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Better than mere attraction – adhesive properties of skin secretion in the Common Rain Frog, *Breviceps adspersus*

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Abstract. Frogs of the genus *Breviceps*, distributed in Eastern and Southern Africa, have short arms, a rounded body shape, and show significant sexual dimorphism in body size. Consequently, the much smaller male is unable to effectively clasp the female during amplexus. Instead, mating pairs are bonded together by an adhesive skin secretion. While both sexes produce the sticky secretion in defence, it is not clear whether the male or female frog, or both, produce the glue during amplexus. Furthermore, there is no basic information on the physical properties of the adhesive secretion in *Breviceps*, such as its adhesive strength, differences in adhesive strength between the sexes, and adhesion duration. In this study, we used an electrical stimulator to induce the release of the adhesive secretion and demonstrated that it is produced by both male and female *Breviceps adspersus* individuals. Additionally, the adhesive strength of the frog secretion was measured for different adhesion durations. The results showed that the adhesive strength was maximal at 1–3 h (median: 8.12 N/cm^2 for females at 1 h duration and 7.20 N/cm^2 for males at 3 h), decreased significantly after one day (0.85 N/cm^2 for females at 24 h and 0.41 N/cm^2 for males) and almost disappeared after three days. A comparison of adhesive strength between the sexes showed few statistically significant differences, suggesting that both male and female secrete the same glue substance. The question of the precise origin of the adhesive secretion during amplexus is discussed.

Key words. Amphibia, Anura, Brevicipitidae, amplexus, adhesive secretion, adhesion strength, Rain Frog.

Introduction

The genus *Breviceps* (Anura: Brevicipitidae), commonly referred to as Rain Frogs, currently consists of 20 nominal species (Frost 2021), mainly found in the arid to semiarid climates of eastern and southern Africa. All known *Breviceps* species are subterranean, spending the dry season underground and emerging above ground in the rainy season to feed and breed (Du Preez & Carruthers 2017). Rain Frogs have a characteristic external morphology, with short arms and a rounded body (Fig. 1A and Minter 1999). In addition, many species show sexual dimorphism, with larger females and much smaller dark throated males (Du Preez & Carruthers 2017). *Breviceps* males are unable to clasp females effectively during amplexus (Duellman 2003). Instead, an adhesive skin secretion enables the sexes to adhere to each other (Fig. 1). A number of amphibians produce sticky secretions that are used in defence against predators (cryptobranchid, hynobiid, and plethodontid salamanders and brevicipitid, microhylid, and limnodynastid frogs; Evans & Brodie 1994, Tyler 2010, von Byern et al. 2017). Adhesive amplexus is known from the families Brevicipitidae (*Breviceps*), Hemisotidae (*Hemisus*) and Microhylidae (*Gastrophryne*, *Kaloula* and some members of the Asterophryinae (Inger 1954, Fitch 1956, Zweifel 1972, Visser et al. 1982, Kyle & Du Preez 2020).

Breviceps species have, for some time, been known to use an adhesive secretion during amplexus (Rose 1962). However, there are only a few, somewhat vague, descriptions of the characteristics of the *Breviceps* glue. It has been reported that when the amplexing pair is forced apart, the skin of the female is injured at the adhesion site (Poynton 1964) and that the pair remain in amplexus for three days (Wager 1965). There has also been some debate as to whether the male or female frog secretes glue. Jurgens (1978, 1979) provided evidence that males produce glue

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during amplexus, while Visser (1979) argued that both sexes produce the adhesive secretion during amplexus; recent authors (Du Preez & Carruthers 2017) have also adopted the latter theory. Based on histological observations of another *Breviceps* species, *B. gibbosus,* Visser et al. (1982) noted differences between males and females, in the distribution of adhesive glands but did not investigate the quantity of adhesive secretion produced by the various areas of skin in which the glands occur.

In addition to the above questions related to sex differences, information on the adhesive properties of *Breviceps* adhesive secretion is sorely inadequate. Specifically, the physical characteristics of the secretion, such as the adhesive retention time and physical adhesive strength, have never been investigated. Visser (1979) speculated that male and female *Breviceps* produce different types of secretion which, when mixed together, form a strong adhesive, as in epoxy glues. However, this epoxy hypothesis has not yet been tested.

In this study, we aimed to obtain basic information on the *Breviceps* adhesive secretion. Specifically, using electrical stimulation, we located the areas of skin that produce this secretion in males and females of *B. adspersus* Peters, 1882, a common species of the genus *Breviceps*. In addition, the adhesive properties of *Breviceps* skin secretion were investigated in terms of duration, maximum adhesive strength, and whether differences in these properties exist between males and females. The epoxy hypothesis is discussed based on our results.

Materials and methods

Animals

In this study, we used 10 male and 10 female *B. adspersus*. These frogs were collected at two urban sites in Polokwane, Limpopo Province, South Africa (23°54'21" S, 29°28'12" E and 23°53'09" S, 29°27'54" E) during the breeding season (December 2017). We performed the following experiments with live animals after obtaining approval from the animal experimental ethics committees.

Collection of the secretion

To collect the adhesive secretion from the frogs, we used a transcutaneous amphibian stimulator (TAS; Grant & Land 2002). TAS and the following procedure did not injure the frogs. First, we anaesthetized the frogs by immersing them in a 0.1% w/v (1,000 ppm) aqueous solution of MS-222 (Tricaine methanesulphonate, Sigma-Aldrich Japan, Tokyo, Japan) for approximately 15 min. After confirming the anesthetic had taken effect, we stimulated the skin of each frog (abdomen, chin–chest, and dorsal areas, separately) with the TAS electrode (Fig. 2A) using the following settings: pulse width = 2 ms, frequency = 80 Hz, and voltage = 12 V. Immediately after transcutaneous stimulation, a milky white secretion was observed on the dorsal skin of the frog (Fig. 2B); secretions from the skin of the abdomen and chin–chest areas were not easily seen, but could be detected by touch.

Test for adhesive strength

To measure the adhesive strength of the secretion from the dorsal skin of both male and female frogs, we used a tensile strength indicator (FGJN-2, Nidec-Shimpo Co., Tokyo, Japan) and several self-made tensile testers (Fig. 2C). The tester consisted of a 3 × 3 × 30 cm length of common cedar, to which an equally spaced series of five acrylic plates, each with a surface area of 1 cm², were firmly attached. Separate acrylic plates of the same dimensions, each firmly attached to a hook, were prepared. This apparatus is highly portable and allowed us to conduct experiments in the field.

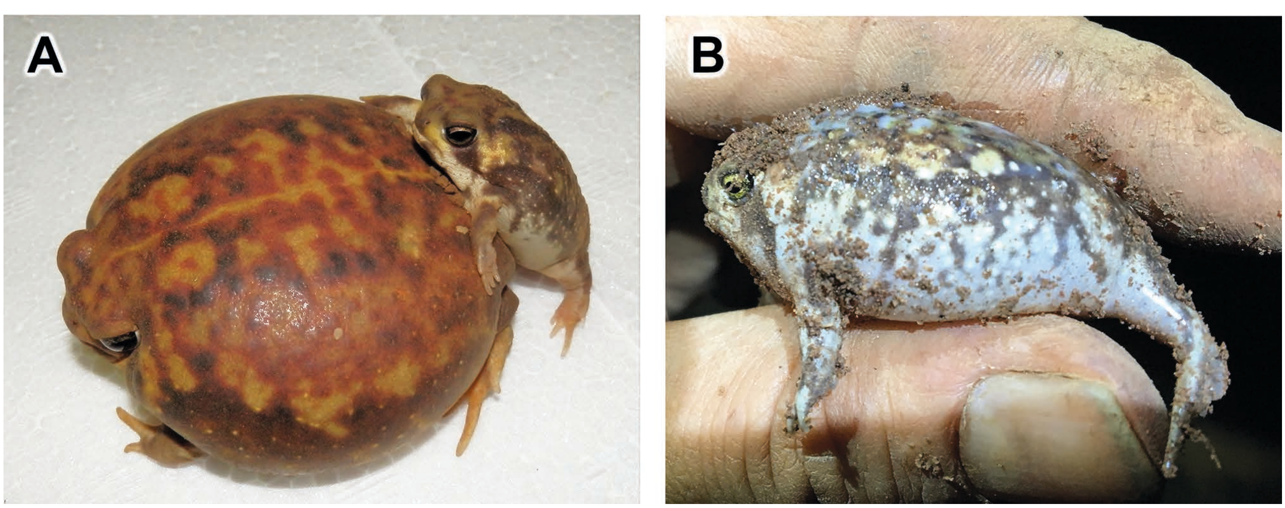

Figure 1. *Breviceps adspersus.* (A) Glued mating pair of *Breviceps adspersus*. (B) Male individual secreting adhesive secretions in alarm.

The test was carried out by smearing adhesive secretion onto the free surface of one of the separate acrylic plates attached to a hook, and then quickly superimposing it onto one of the five acrylic plates attached to the length of cedar wood (Fig. 2D). Although we did not weigh the adhesive secretion used in all adhesion trials (see Discussion section), the average weight of the adhesive secretion used in five trials was 6.3 (± 1.7 standard deviation) mg.

The tensile strength (N/cm²) of the adhesive secretion was tested after seven specific time periods had elapsed, i.e., 15 and 30 mins, 1, 3, 6, 24 and 72 hrs. The length of cedar wood was suspended horizontally by resting its ends on the edges of two adjacent tables, with the acrylic plates facing downwards. To measure the adhesive strength of the secretion after the required time had elapsed, the hook of the tensile strength meter was engaged with the hook of the acrylic plate and pulled vertically downwards until the two acrylic plated separated (Fig. 2D). Two sets of time-trials were conducted for each individual, i.e., a total of 14 (2×7) tests. If the acrylic plates were unintentionally misaligned or separated before the test was completed, the test was treated as missing data (32 cases of all 280 tests; see Supplementary Table S1). The results are summarised in a box plot using R statistical software ver. 4.0.0 (R Core Team 2020).

To investigate whether the mixing of male and female glue-like mucus affects the adhesive strength (as suggested by Visser et al. 1982), the following experiment was carried out. The secretion from the chin–chest area of the males and the dorsal area of the females, which come into contact during the actual amplexus, were smeared onto separate acrylic plates (each plate has a hook). The quantity of secretion produced by the chin–chest region of males was very small (less than 1 mg) but its presence was confirmed visually. The plates were pressed together manually, using the fingers, and after 3 h, the adhesive strength of the secretion was measured by attaching one hook to a horizontal metal bar, and the other to the hook of the tensile strength me-

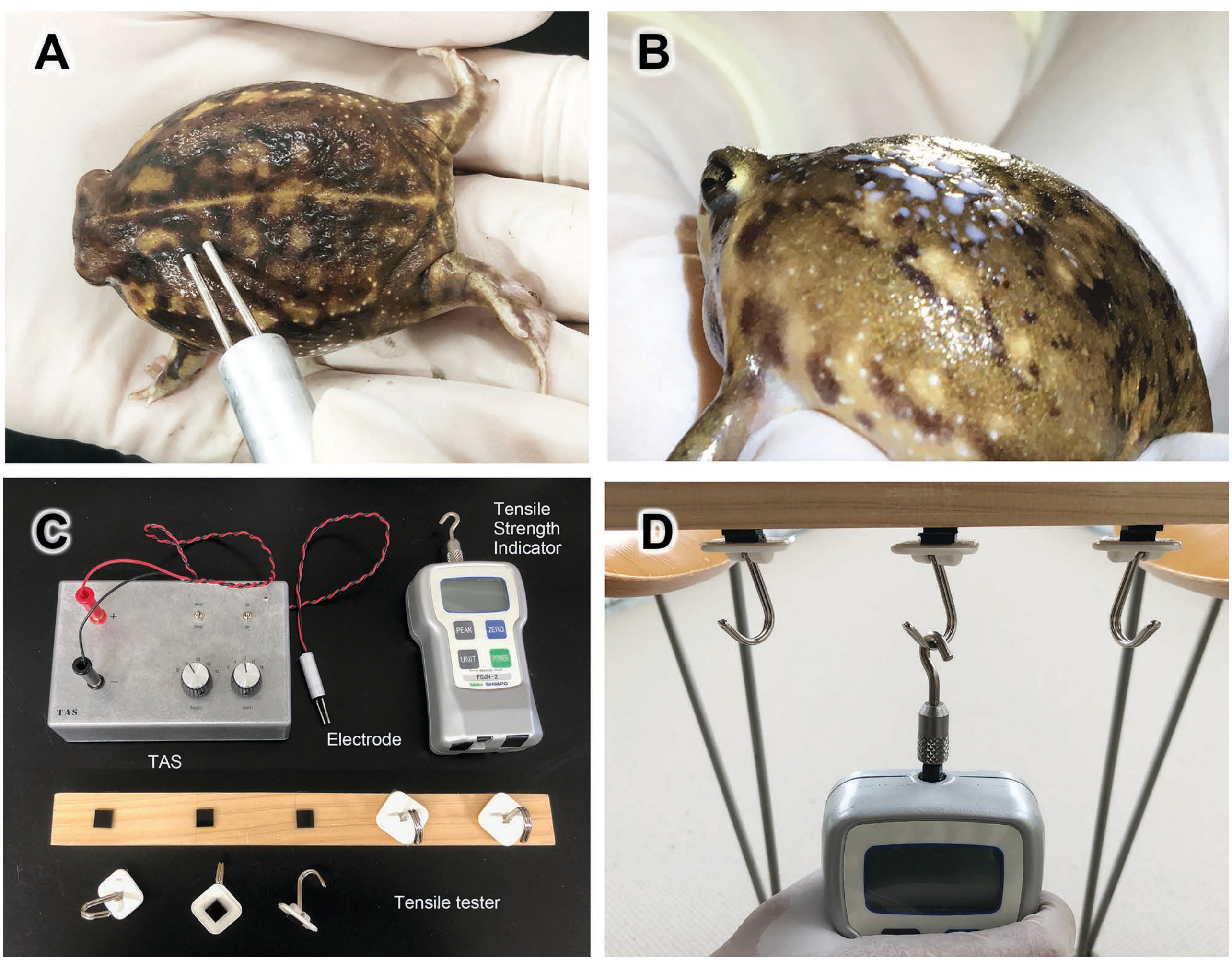

Figure 2. Artificial stimulation of adhesive secretions by TAS, and equipment for measuring adhesive strength. (A) Stimulation by TAS (Transcutaneous Amphibian Stimulator). (B) Adhesive secretion on dorsal skin of female frog. (C) Equipment for measuring adhesive strength of skin secretions. (D) Measurement of adhesive strength.

ter, pulling vertically downwards, and measuring the force required to separate the two acrylic plates. We performed 10 adhesion trials with samples of secretions from seven males and eight female specimens.

Statistical analysis

Mann-Whitney U (M-W-U) tests were performed using R software for 1) the difference in tensile strength of the adhesive secretion between the males and females after each elapsed-time period, 2) the difference in tensile strength among the seven elapsed times tested, and 3) the difference in tensile strength between the mixed adhesive secretions of males and females and unmixed secretions (after 3 h had elapsed). For test 2), we applied Bonferroni correction, since this is a multiple comparison.

Results

Adhesive secretion of *Breviceps adspersus* released using transcutaneous stimulation

Transcutaneous stimulation using TAS resulted in the immediate release of the adhesive secretion from the dorsal skin glands of all *B. adspersus* individuals tested. The secretion retained its thin milky consistency for some time on the skin, but solidified rapidly when transferred to other substrates, specifically plastic, paper, wood, aluminium alloy, and stainless steel. This made it difficult to weigh the secretion accurately and regulate the amount used in the adhesive strength tests. In many cases, the adhesive secretion from other areas of the skin was not easy to see and was instead detected using the experimenter's finger for the tactile sensation. The adhesive secretion was detected in all 10 females and eight out of 10 males in the chin–chest area. However, transcutaneous stimulation failed to induce the release of adhesive secretion from the abdominal skin of either female or male frogs.

Tensile strength of the adhesive secretion

We measured the tensile strength of this secretion from the dorsal skin of the 20 frogs after seven different adhesion periods (Table 1). The resultant 248 measurements (excluding 32 unintentional misaligned trials from all 280 tests) are summarised in Table 1, and the box plots for each sex and for each period are shown in Figure 3. The maximum adhesive strength, 43.14 N/cm^2, was measured for a female after 24 h of adhesion (Fig. 3 and Supplementary Table S1). The 2nd and 3rd maximum adhesion strengths, 25.36 N/cm^2 and 21.28 N/cm^2 were measured in females after 6-h of adhesion. The maximum adhesion strength in males was 20.03 N/cm^2 (after 3 h of adhesion), followed by 15.87 N/cm^2 (6 h of adhesion) and 15.79 N/cm^2 (1 h of adhesion). However, these high adhesive strengths are exceptional values, and are treated as outliers in the box plot (Fig. 3). In most cases, the tensile strength of the adhesive secretion of *B. adspersus* was much weaker, with a median (and mean) value of 3.91 (4.89) N/cm^2 for all 248 measurements (Table 1). The median (and mean) values of adhesion for the entire male and female trials (N = 116 and 132) were 3.80 (5.12) and 4.05 (4.69) N/cm^2, respectively, with no significant difference between them ($P = 0.689$, M-W-U test). A significant difference in adhesion strength between males and females was observed at 15 min ($P = 0.008$; Fig. 3), but no significant difference in adhesion strength between males and females was observed in the remaining six adhesion periods. Overall, the tensile strength of the *B. adspersus* adhesive secretion seems to be similar between the sexes.

Table 1. Median and mean values of adhesive strength of *Breviceps adspersus* adhesive secretions at each adhesion duration. Total means both males and females. Mix indicates the trial of the mixing female's and male's glue secretion.

	Adhesion duration	Median (N/cm^2)	Mean (N/cm^2)
Total	15 min	4.29	4.45
	30 min	4.29	4.08
	1 hour	6.89	7.89
	3 hours	6.39	6.96
	6 hours	3.32	5.74
	24 hours	0.52	3.10
	72 hours	0.00	1.30
	All time	3.91	4.89
Female	15 min	3.24	2.95
	30 min	4.24	3.85
	1 hour	8.12	8.65
	3 hours	4.78	6.64
	6 hours	3.68	6.39
	24 hours	0.85	4.86
	72 hours	0.00	1.58
	All time	3.80	5.12
Male	15 min	4.92	5.49
	30 min	4.39	4.23
	1 hour	6.03	7.20
	3 hours	7.20	7.27
	6 hours	3.32	5.22
	24 hours	0.41	0.84
	72 hours	0.00	1.04
	All time	4.05	4.69
Mix	3 hours	7.85	7.80

Change in adhesion strength over time

We compared the tensile strength of the adhesive secretion of *B. adspersus* for seven different adhesion time periods. For the entire sample (females + males), the median value

Table 2. Difference in adhesion strength between adhesion periods and the significance of the difference. The bold numbers in the first column show the median values (N/cm²) of the measured adhesive strengths of the females and males and females for each adhesion duration. * and ** represent P < 0.05 and 0.01, respectively, in Mann-Whitney U test with Bonferroni correction showing the significance of the differences among adhesion times compared.

	Median	15 m	30 m	1 h	3 h	6 h	24 h
15 m	4.29						
30 m	4.29	1.000					
1 h	6.98	0.006**	0.000**				
3 h	6.39	0.868	0.393	1.000			
6 h	3.32	1.000	1.000	0.189	1.000		
24 h	0.52	0.001**	0.000**	0.000**	0.000**	0.067	
72 h	0.00	0.000**	0.000**	0.000**	0.000**	0.000**	0.008**

of adhesion strength reached its maximum at 1 h duration (6.98 N/cm²). This value did not significantly differ from those in 3- and 6-h cases but was significantly greater than those in 15- and 30-min and 24- and 72-h cases (P < 0.05 by M-W-U tests with Bonferroni adjustment; Table 2).

For females, the median value of adhesive strength was greatest after a period of 1 h (8.12 N/cm²; Table 3), and significantly greater than the values measured after 15, 30, and 72 h (P < 0.05). In males, the median value of adhesion strength was greatest after 3 h (7.20 N/cm²; Table 3) and significantly greater than that at 24 and 72 h (P < 0.05). The adhesion strength of the secretion in males at 24 and 72 h (0.41 and 0.00 N/cm²) was significantly weaker than that measured after 15 min, 1 h, and 3 h (P < 0.05). The adhesion strength in females at 24 h was also lower (0.85 N/cm²), but not significantly different. Significant differences were detected in adhesion strength in the secretions of females at 72 h compared with 1 h and 3 h (P < 0.05).

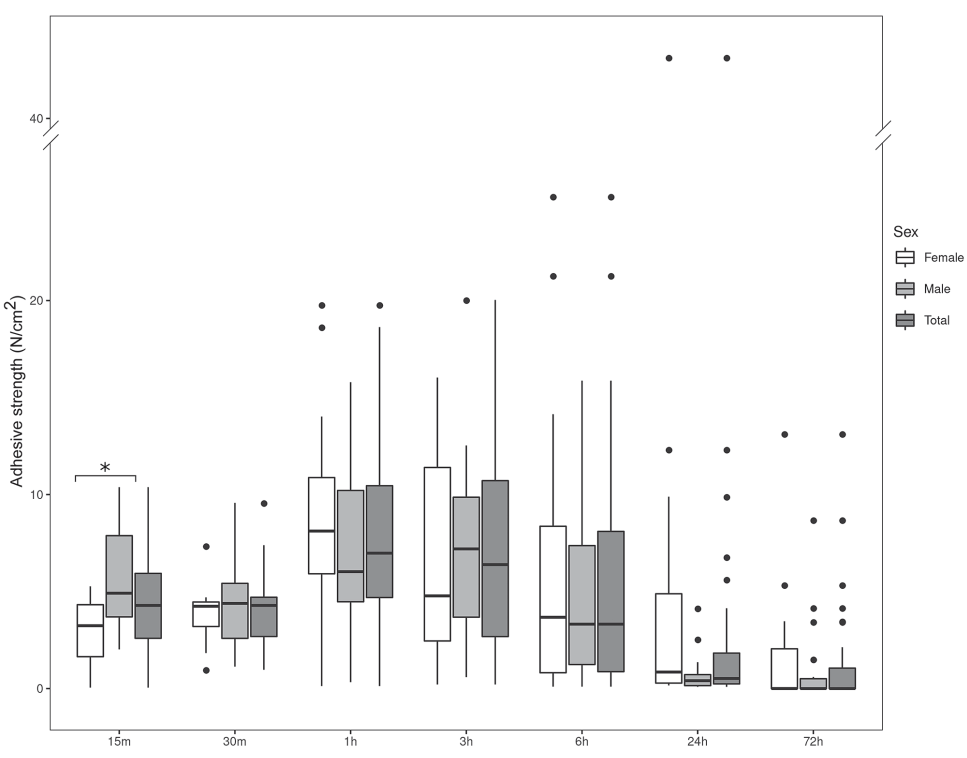

Figure 3. Box plot representation of adhesive strength of *Breviceps adspersus* skin secretions. The vertical axis shows the adhesion force (N/cm²). The measured adhesive strengths are separately shown at each adhesion duration (7 distinct durations between 15 minutes and 72 hours). The adhesive strengths of females, males, and total individuals are separately represented too. Dots indicate outlier adhesion strengths. Within each adhesion duration, the significant difference (* P = 0.008 by Mann-Whitney U test) of adhesive strength is only observed between female and male at 15 min duration.

Table 3. Difference in adhesion strength between adhesion periods in each female and male and the significance of the difference. The bold numbers in the first row and column show the median values (N/cm²) of the measured adhesive strengths of the adhesive secretions of males and females for each adhesion time. The upper-right and lower-left denote the P-values of the difference in adhesive strength between adhesion durations separately calculated from females and males, respectively. * and ** represent P < 0.05 and 0.01, respectively, in Mann-Whitney U test with Bonferroni correction.

	Median (Male)	15 m	30 m	1 h	3 h	6 h	24 h	72 h
Median (Female)		3.24	4.24	8.12	4.78	3.68	0.85	0.00
15 m	4.92		1.000	0.036*	0.349	1.000	1.000	0.836
30 m	4.39	1.000		0.049*	1.000	1.000	1.000	0.559
1 h	6.03	1.000	0.198		1.000	1.000	0.524	0.003**
3 h	7.20	1.000	0.404	1.000		1.000	1.000	0.000**
6 h	3.32	1.000	1.000	1.000	1.000		1.000	0.379
24 h	0.41	0.013*	0.120	0.003**	0.005**	0.110		1.000
72 h	0.00	0.001**	0.049*	0.009**	0.002**	0.000**	1.000	

In summary, the adhesive strength of the glue secretion tended to be weak at short adhesion durations (15–30 min), increased at 1–3 hours, then weakened and was negligible after 72 h (Fig. 3). This tendency was observed in all tested categories.

Effect of mixing female and male adhesive secretions

We compared the adhesive strength of skin secretions in females, males, and their combined secretions after 3 h. The median values of the tensile strength of male and mixed samples were similar to each other (7.20 N/cm² and 7.85 N/cm², respectively), while that in females was slightly lower (4.78 N/cm²) (Table 1). However, there were no significant differences among the median values of the three samples (P > 0.05, M-W-U test).

Discussion

In this study, we artificially collected adhesive secretions using electrical stimulation and investigated the properties of *Breviceps* glue. The margin of error in our measuring methods was negatively impacted by the following factors: 1) the necessity of carrying out the experiment in the field and 2) the rapid hardening of the adhesive secretion after it is transferred from frog skin to another substrate, made it impossible to carry out manipulations such as standardising the amount of adhesive secretion. Furthermore, it should also be mentioned that the proportion of the constituents of the adhesive secretions were not always constant, from individual to individual, specifically with regard to the water content of the adhesive secretion, which varies between frog individuals, and even among different attempts to obtain secretions from the same individual (Kurabayashi et al., unpubl. data). Another factor that should be considered is that the adhesive secretions in *Breviceps* contain a large number of proteins (Kurabayashi et al., unpubl. data), and any one (or a few) of these proteins could be the key (adhesive) ingredient of the secretion, as is the case in an Australian *Notaden* species (Graham et al. 2005). The amount of the adhesive protein(s) in the secretions could also vary from one individual to another. For these reasons, it is unlikely that standardisation of the amount of adhesive secretion used in the tests for tensile strength would result in a reduction of the margin of error. Instead, we attempted to reduce experimental error by increasing the sample size and the number of tests performed (total N = 248).

There has been some controversy as to which sex produces the adhesive secretion during amplexus in *Breviceps* (Jurgens 1978, 1979, Visser 1979). Jurgens (1978) published a photograph of a captive *B. adspersus* adhering, by the skin of its chest, to the back of a *Tomopterna delalandii*, which demonstrated that male *Breviceps* do produce an adhesive secretion during amplexus. He further noted that "At this stage it is not known if the female also produces glue." During the present study, we observed a captive *Arthroleptis stenodactylus* that had been amplexed by a male *Breviceps* sp. (Fig. 4), as well as several instances in which male *B. adspersus* amplexed other conspecific males. This phenomenon is also known in *B. gibbosus*, where 6 males were observed adhering to one another in various positions, forming a writhing, rounded mass (Harrison & Minter, 2004)

Visser et al. (1982) carried out a histological study of the skin of *B. gibbosus*, which revealed the presence of unicellular and multicellular mucous-producing glands, granular (serous) holocrine glands and apocrine glands similar in structure to the adhesive breeding glands of *Gastrophryne carolinensis* described by Conaway & Metter (1967) (also see Siegel et al. 2008). They found that in females, both holocrine and apocrine glands were very abundant in the skin of the lower back, while in males, the skin of the sternal area was rich in holocrine glands but had fewer apocrine glands. Since these are the areas of skin that are in contact during amplexus, they concluded that the holocrine granular glands of both males and females contributed to the adhesive secretion produced during amplexus. They did not speculate on the function of the secretion produced by the apocrine glands. They also noted other differences between males and females in the distribution and abundance of skin glands: holocrine and apocrine glands were present, but less common, in the dorsum of males, while the sternal area of females contained apocrine but no holocrine glands. In the present study of *B. adspersus* no significant differences were found between males and females regarding the distribution of the glands that produced the adhesive secretion. Both males and females could produce large quantities of adhesive secretion on the dorsum, and smaller quantities in the gular and sternal areas. Adhesive secretions were not produced by the abdominal skin of either males or females following transcutaneous stimulation.

In the present study, at least in *B. adspersus*, we clearly showed that both sexes produce adhesive secretions, which support Visser's hypothesis. However, we were unable to resolve the question as to which (or both) sexes release these secretions during amplexus, because the secretions were released under artificial stimulation. Our observations do not rule out the possibility that female adhesive secretions are used in the formation of glued pairs in these frogs, but it seems certain that amplexus is possible by means of the adhesive secretion of the male alone.

Visser (1979) proposed an interesting idea that female and male *Breviceps* produce different adhesive secretions which, when mixed, exhibit strong adhesive strength like that of commercial epoxy glue (also see, Jurgens 1979, Visser et al. 1982). In the present study, however, there was no significant difference in the adhesive strength of the secretion between male and female *B. adspersus* in most tests (except for the measurements at 15 min of gluing). Furthermore, no significant difference was found in the adhesive strength of the mixed secretions of males and females when compared with that of unmixed samples. There is a possibility that the quantity of secretion from the male chin–chest area was so small that the mixing effect was insignificant. However, this study, the first experimental attempt to test Visser's epoxy hypothesis does not support his idea. Rather, our results strongly suggest that both male and female *Breviceps* produce (possibly identical) adhesive substance(s) with similar adhesive properties.

In both *Breviceps* sexes, the median value of adhesion strength of the adhesive secretions was maximal after 1–3 hours, less than 1.0 N/cm² after 24 hours, and lost its adhesive properties by 72 hours. Wager (1965) believed that the duration of amplexus in a *Breviceps* pair is approximately 3 days. In this study, most newly amplexed *Breviceps* pairs separated after 2–3 days. Thus the duration of adhesion observed in our samples correlates with the duration of actual observed amplexus.

An amplexed *B. adspersus* pair burrows downwards into the soil to create a nest cavitiy for egg laying (Wager 1965, Minter 1999). The smaller male remains attached to the female during this period since the secretion retains its adhesive properties for up to 3 days. However, we found that the water content of the secretion affected its adhesion strength over time. Our adhesion experiments were mainly carried out under ambient relative humidity levels of 50–80% because we were unable to carry out our research under controlled humidity conditions. However, on one oc-

Figure 4. Male *Breviceps* sp. in adhesive amplexus with *Arthroleptis stenodactylus.*

casion, when the experimental apparatus was left at a relative humidity of 20%, all 20 acrylic plate pairs joined by the adhesive secretion separated during the night (16 h). This observation suggests that the decrease in water content due to evaporation leads to a natural loss of adhesive strength of the skin secretion and amplexed pairs separate without the need for any special detachment mechanism.

The greatest tensile strength of the *B. adspersus* adhesive secretion measured in this study was 43.14 N/cm², observed in a test of the secretion of a female secretion after 24 hours (Supplementary Table S1). However, this is a quite unusual case as the maximum mean values were 8.65 N/cm² for females at 1 h and 7.27 N/cm² for males at 3 h (Table 1). Although the measurement method is different, these median values of the tensile strengths of *B. adspersus* adhesive secretions are second only to those of *Dyscophus* frogs which have the strongest adhesive strength among the 12 amphibian glues measured by Evans & Brodie (1994) (*D. antongilii*: 9.2 N/cm²; *D. guineti*: 10.4 N/cm²) and like that of *Notaden bennetti* (7.8 N/cm² in the moist glue; Graham et al. 2005). Thus, the adhesive strength of the *B. adspersus* adhesive secretion is within the range of that of other known amphibians and relatively high.

The median and mean values of all 248 measurements (3.91 and 4.89 N/cm²; Table 1) of the adhesion strength of the adhesive secretion are converted to 398.71 and 498.64 gf/cm² in the gram-force unit system. The average body weight (± standard deviation) of *B. adspersus* males (8 individuals whose body weight was measured) was 7.37 ± 1.08 g, and the area of contact between amplexing pairs (chin–chest–arms in males) was more than 1 cm². Therefore, the adhesive strength is more than sufficient to securely attach the male body to the female dorsum. The presence of adhesive glands on the dorsum of the male is probably due to the function of the secretion in defence against predators. Defensive adhesive secretions are known from many amphibians (Evans & Bordie 1994), including *Balebreviceps hillmani* and *Callulina* spp., members of the family Brevicipitidae (Largen & Drewes 1989, Measey et al. 2009, Malonza 2008). If the adhesive secretions of *Breviceps* and the other brevicipitid genera have the same origin (acquired in a common ancestor of the family), then the secretions produced on the back of *Breviceps* males could be regarded as a remnant of the ancestral condition (having a defensive role). The reason why the adhesive strength of the *Breviceps* skin secretion is more than sufficient for amplexus may be that its original purpose was to defend against predators and its role in amplexus is a secondary function. It is known that *Breviceps* produces the adhesive secretion when alarmed (Du Preez & Carruthers 2017), and, albeit infrequently, we found that a few *Breviceps* individuals produced the secretion when they were collected (Fig. 1B).

In this study, we obtained many insights into the adhesive properties of the skin secretions of *B. adspersus*, which have not been clarified previously. However, investigations on the chemical properties of the adhesive secretions, its main constituents, and the genes responsible for producing these secretions should be further researched. In addition, although we have reported the *Breviceps* adhesive secretion in only one species here, our investigations of other species have shown that the adhesive strength of the secretion differs among *Breviceps* species, and that some species produce secretions with much stronger adhesive power. Clarifying the environmental and/or genetic factors which determine the adhesive strength of these secretions in *Breviceps* will be an interesting research topic for future investigations.

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Supplementary data

The following data are available online:

Supplementary Table S1. Data of adhesive strength of all individuals and duration of adhesion.