

## Behavioral mechanisms of male sterilization on plateau pika in the Qinghai-Tibet plateau

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### ABSTRACT

Fertility control is an alternative non-lethal method in the management of rodents. Previous modeling suggests that the efficacy of male sterilization depends on mating systems of animals, but behavioral mechanisms of male sterilization have not been investigated. Here we investigated the behavioral mechanism of the sterilant quinestrol in reducing the fertility of plateau pika (*Ochotona curzoniae*) inhabiting the Qinghai-Tibet plateau. Male pikas treated with quinestrol showed reduced aggression compared to control males, but they showed significantly higher levels of territorial behavior such as long-calls and long-chases. Levels of long-call and long-chase were negatively correlated with the number of newborn pikas in the family. Single-baiting of quinestrol effectively sterilized male pikas and reduced the pregnancy rate of female pikas; this was likely achieved by increased territorial behavior of sterilized pikas which resulted in unsuccessful invasions by fertile adult male pikas. Our study reveals a novel behavioral mechanism, increased territoriality in sterilized males, in the fertility control of plateau pikas.

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### 1. Introduction

The plateau pika (*Ochotona curzoniae*) is a dominant small mammal in the alpine meadow ecosystems across the Qinghai-Tibetan plateau, China. During the breeding seasons, plateau pikas live in family groups composed of several adult males and females (Qu et al., 2008; Smith and Wang, 1991; Dobson et al., 1998). All members in the same family group share a territory and burrow system (Wang and Dai, 1990). There is often one dominant male in each family which has greater mating chances than other males (Yin et al., 2009). The litter size of plateau pikas is 3–6 young and juveniles usually leave burrows to forage approximately 20–30 days after birth (Wang and Smith, 1989; Smith and Wang, 1991). Juvenile pikas can grow nearly as large as their parents by the end of the breeding season in June, but are still immature (Qu et al., 2008). Most juvenile pikas would die by next year after mating, but about 15% of them can survive and breed again by the third year. During the breeding season, plateau pikas rarely disperse (Dobson et al., 2000), however in the non-breeding season, a male-biased

dispersal will increase and family structure changes (Smith and Ivins, 1983; Smith and Wang, 1991).

Plateau pikas are considered a keystone species within the plateau ecosystem (Lai and Smith, 2003; Smith and Foggin, 1999) but also as pests across the Qinghai-Tibetan plateau because they can cause serious damage to grasslands when their population densities are high (Liu et al., 1980; Xia, 1984; Xin, 2008). In recent decades, due to rapid increases in livestock such as sheep (*Ovis aries*) and yaks (*Bos grunniens*), the grasslands have become heavily degenerated from over-gazing. This is thought to facilitate growth of the pika population (Fan et al., 1999). Traditional culling with chemical rodenticides and biological toxins (e.g. botulin toxin C) has been used to control pika populations, but it presents risks to non-target animals and contributes to a loss of biodiversity (Lai and Smith, 2003). The pika population can recover very quickly after lethal poisoning (Pech et al., 2007; Liang, 1981). Thus, it is necessary to develop non-lethal methods for managing animal damage and maintaining healthy ecosystems.

According to ecological modeling, fertility control can slow population recovery because infertile individuals continue to occupy the territory and access resources and mates (Shi et al., 2002; Zhang, 2000; Caughley et al., 1992). Fertility control can reduce reproduction and population density through sterilization of both sexes. Infertile females directly contribute to a decrease in population growth, while infertile males contribute to decreases indirectly

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through females. The efficiency of male sterilization depends on the mating systems of animals (Zhang, 2000). For mating systems with more males (i.e. promiscuity, polyandry or polygynandry), a high proportion of males need to be sterilized to suppress population growth. However, for mating systems with one male (e.g. monogamy or polygyny), one sterilized male can cause infertility in one or more females. Mating systems, which are partially decided by behavioral styles (Stapley and Keogh, 2005; Ostfeld, 1986), are critical for modeling the effects of fertility control.

Territorial behavior is also important for the efficacy of fertility control. If infertile individuals exhibited lower frequency of territorial behavior, fertility control usually cannot reach to ideal results. Lack of territorial behavior will increase the immigration in the population, elevate the chance of the mating between two survival fertile individuals, and finally weaken the efficacy of fertility control. For male fertility control, keeping a high frequency of territorial behavior is more than important to maintain the efficacy, because in theory, one fertile male can mate with many normal females to fight against fertility control effectively. It is therefore necessary to explore behavioral responses, especially territory behaviors, after the application of fertility control.

Our previous research has indicated that the synthetic estrogen quinestrol is very effective at reducing infertility in males of many rodent species (Huo et al., 2007; Liang et al., 2006; Wan et al., 2006; J. Zhang et al., 2004; Z. Zhang et al., 2004; Zhang et al., 2005, 2006; Zhao et al., 2007). A large-scale field test indicated that application of this compound can cause high levels of infertility in male plateau pikas in the Qinghai-Tibetan plateau, and this leads to low pregnancy rates for female pikas (Liu et al., *in press*). Low pregnancy rates mean fewer newborn pikas, which may induce adult male pikas to continue protecting their mating partners, causing the increase of aggressive and territorial behaviors. However, as an estrogen, quinestrol can suppress testosterone production and therefore reduce the aggressive behavior (Bahrke et al., 1996; O'Donnell et al., 2001). A lower frequency of aggressive behavior may cause higher rates of immigration between family groups and impair the infertility efficacy of quinestrol. The purpose of this study is to explore the effects of fertility control on aspects of male behavior of populations of plateau pikas. Specifically, we ask if either infertility in male pikas or quinestrol itself contributes to low pregnancy rates in females through behavioral change, and if family structures change via either pure social (behavior only exhibited in family group) or individual behavior when dominant males become infertile.

## 2. Methods and materials

### 2.1. Study site

Experiments were conducted near Dawu town (E 100.2821°, N 34.4292°), Maqin county, Qinghai, China. The study site is a typical meadow ecosystem of the Qinghai-Tibetan plateau. Altitude is 3700–3800 m above sea level. Two experiments were conducted at the study site. Experiment I was carried out in natural conditions to investigate changes in behavior and social structure of pikas. Experiment II was conducted in enclosures to investigate factors affecting changes in behavior and social structure of pikas. In all experiments, animal care and treatment were conducted by following regulations of the Northwest Institute of Plateau Biology/Institute of Zoology, Chinese Academy of Sciences.

### 2.2. Experiment I: changes in territorial behavior and family structure

This experiment was designed to test whether sterilization would change territorial behavior in male pikas. In 2008 we

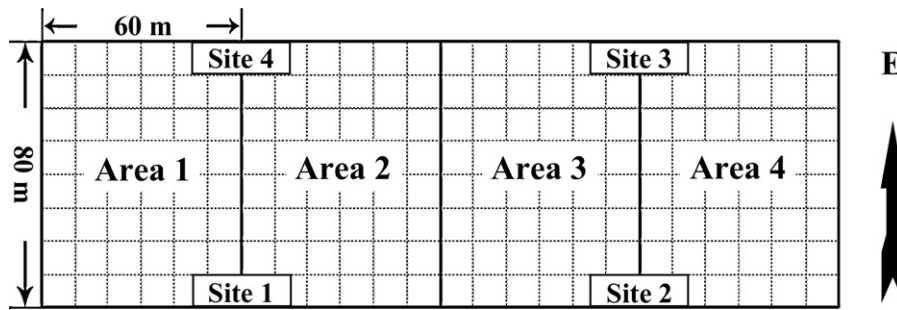
conducted a fertility control experiment on pikas in a large area of 6666 ha near Dawu in collaboration with local pastoral officers using oat bait containing 0.005% quinestrol. In our former research, application of baits containing 0.005% quinestrol has been proved to be an effective in causing infertility of male pikas for over 2 months, while did not affect the fertility of the females (Liu et al., *in press*). We selected a plot (100 m × 100 m) in the middle of the quinestrol treatment area (quinestrol-treated group: Qui), and another plot (100 m × 100 m) in a normal area (control group: Con) to observe territorial behaviors in 10 family groups of pikas. There was no replication for Experiment I, and family groups were considered to be the experimental units. All adult pikas were caught and ear-tagged in early May. Male and female pikas were dyed differently for easy visual identification. Males were dyed black on the head, and females were dyed black on the back. Observations were conducted five times (May, late May, early June, mid-June and late June) covering most of the pika breeding season.

We measured the frequency of long-calls and long-chases, two common territorial behaviors of pikas (Svendson, 1979; Smith et al., 1986). A long-call is composed of a series of short calls made by males. Pikas usually “stand up” when they are calling, so we can easily observe from where calling occurs. During breeding season there are measurable increases in the frequency of long-calls (Wang and Dai, 1990; Smith et al., 1986). Long-chases are another type of territorial behavior; pikas will chase invaders out of their territory (Wang and Dai, 1990). The time period of observation for behavior of pikas on the ground was between 8:30 am and 11:30 am when the pikas were most active during the day. The observation lasted 1 h, twice for each day at one of the four corners in both Con and Qui plots separately. A long-call was recorded if the observer heard the call, and the long-chase was recorded if the observer watched a chase occurring in the selected plot. Observations were postponed on bad weather days.

The structure of each family group was observed together with behavior in late May, early June, mid-June and late June. We used telescopes to observe relationships among pikas and considered them to belong to the same family if they showed affiliative behavior (Wang and Dai, 1990; Smith et al., 1986) towards each other. Members belonging to one family group were identified by their unique ear-tags. When a family group was identified its location was marked for future observations. Family members were classified into the following categories: resident adult pikas of the family, newborn pikas of the family, and immigrant adult pikas from outside the family.

### 2.3. Experiment II: factors affecting the territorial behavior of infertile males

This experiment was used to test key factors affecting the territorial behavior of infertile male pikas. We focused on two factors of infertile males and newborn pikas based on our observations in the field. There was also no replication for Experiment II and family groups were considered to be the experimental units. Four enclosures were used in this experiment ( $H \times L \times W = 1.5 \text{ m} \times 80 \text{ m} \times 60 \text{ m}$ ) (Fig. 1). Artificial burrow tunnels were horizontal round holes constructed 20–30 cm below ground, with a diameter of 5 cm. There were two groups of burrow tunnels in each enclosure, and either group was composed of a number of parallel burrow tunnels. Inside either group, any two burrow tunnels next to each other had an interval of 10 m. The two groups were perpendicular to each other, forming squares of  $10 \text{ m} \times 10 \text{ m}$  (Fig. 1). The artificial burrow tunnels were used by pikas as initial nests or shelters to prevent attack from avian predators. In late April 2009, adult pikas were caught near Dawu and released into the enclosures immediately to let them adapt to the new environment. All caught pikas were weighed and ear-tagged. Pikas were marked



**Fig. 1.** Illustration of experimental enclosures. Sites 1–4: observation sites. Area 1–4: four enclosures for Experiments 1–4. Each enclosure contains 80 m × 60 m. Dashed lines indicate artificial burrow tunnels. Each enclosure contains seven 60 m and five 80 m burrow tunnels.

with dye as in Experiment I. A total of 42 pikas were released into each enclosure (equal numbers of males and females). The density is 84 pikas/ha, similar to those in Experiment I (80 pikas/ha in control area and 92 resident pikas in quinestrrol-treated area based on method of ear-tagged recapture). Pikas used the artificial burrows very quickly. All pre-experiment work was completed before 25 April 2009 when pikas began to breed.

The four enclosures were divided into four groups. In Enclosure 1 (Area 1), males were treated with quinestrrol baits (0.005%), and pregnant females were introduced into the enclosure (group 1: Q-I group). This group contained infertile males and newborn pikas produced by the introduced pregnant females, however these females were unable to reproduce a second time due to sterilization of the male pikas. In Enclosure 2 (Area 2), pikas were given control baits. Newborn pikas were removed from the enclosure mid-May (group 2: C-R group) and would appear again in June since both males and females were reproductively normal. The number of newborn pikas was lower compared to the control group (see Enclosure 4 below) due to the removal of newborn pikas in mid-May, but would increase again with the second breeding of normal females. In Enclosure 3 (Area 3), pikas were treated with quinestrrol baits (0.005%) (group 3: Qui group). This group had infertile males and no newborn pikas due to breeding failure. A few newborn pikas were observed because unidentified pregnant females came into the enclosure in May, they were subsequently removed.

Group 3 was used to simulate the results of the quinestrrol-treated plot in Experiment I. In Enclosure 4 (Area 4), pikas were provided normal baits and newborn pikas were not removed (group 4: Con group). Group 4 had normal males and females and was used to simulate the results of the control plot in Experiment I. This design was used to determine the cause of increased male territorial behavior. Quinestrrol-treatment and newborn pikas were the two candidate factors. In Experiment II, group 1 was set as quinestrrol-treatment positive and newborn pikas positive; group 2 was set as quinestrrol-treatment negative and newborn pikas positive; group 3 was set as quinestrrol-treatment positive and newborn pikas negative; group 4 was set as quinestrrol-treatment negative and newborn pikas positive (see Table 1).

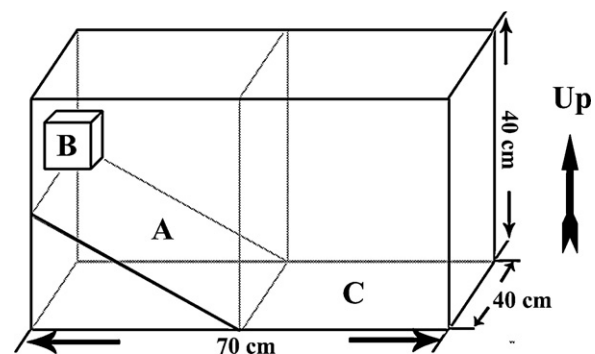
**Table 1**  
Treatment of the four enclosures and territorial behavior in May as compared to control group in Experiment II.

Treatment groups	Q-I group	C-R group	Qui group	Con group
Male sterilization	Yes	No	Yes	No
Presence of newborn pikas	Yes	No	No	Yes
Territorial behavior in May	Lower	Higher	Higher	–

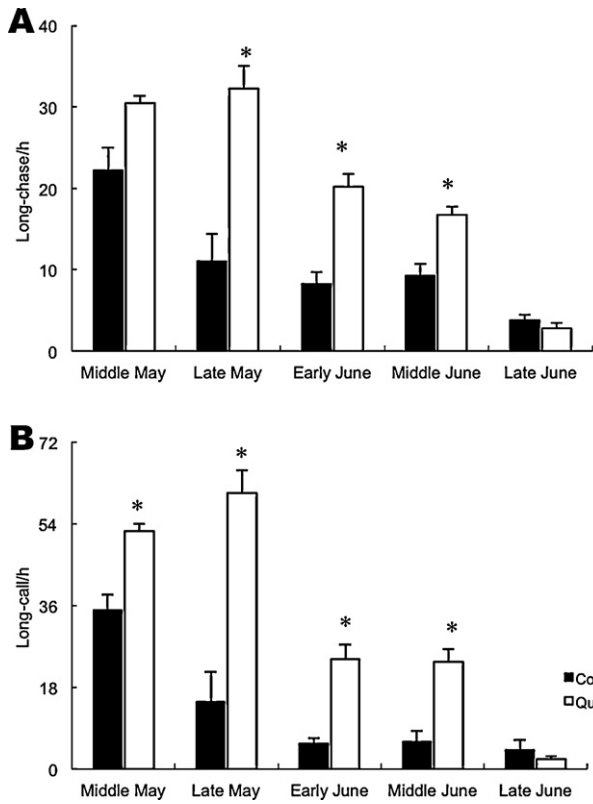
Normal baits were plain oat containing 2% sugar to improve palatability, and the quinestrrol baits were plain oat containing 0.005% quinestrrol and 2% sugar. A total of 1.5 kg of baits was delivered to each enclosure, placed near entrances of the active burrows. The amount of bait per ha in Experiment II was same as that in the Experiment I. A pilot single baiting experiment was conducted on 26 May 2008. Our pre-experiment indicated that one single-baiting of 0.005% quinestrrol would result in complete sterilization of male pikas for the entire breeding season.

Four observation sites were selected as shown in Fig. 1. Each site contained two enclosures. Two observations were conducted for each sampling time at each site. In each enclosure, five family groups were selected in early May 2009 to study changes in territoriality and family structure. They were surveyed in mid-May, late May, early June, mid-June and late June as in Experiment I.

Aggressive behaviors of male pikas from Enclosures 1, 2 and 3 were measured in mid-May 2009. Five groups of pikas (each containing two males and one female) were captured from the same enclosure but not from the same family group. Injured pikas or female pikas in estrus were not used. Before being released into the equipment shown in Fig. 2, three pikas (two males and one female) were placed in a cage (27 cm × 16 cm × 12.5 cm) for 5 min to become familiar with each other. The two male pikas were placed in the right side of the equipment (Fig. 2), while the female was placed in a small box in the left side of the equipment. The two male pikas would then fight each other for the female. The total number of bites by the two males within the observation time was used to define aggression level. The total number of approaches to the female along the incline by the two males was defined as the mating tendency level. Pika behavior was not recorded for



**Fig. 2.** The experimental cage designed for measuring aggressive behaviors of male pikas in the presence of a normal female pika. (A) Smooth slope plate, male pikas climb along the plate to approach female placed at site B. Males cannot stay on the plate long because it is very smooth. (B) Site where the normal female pika is kept. (C) Space for the two introduced male pikas to compete for the female at site B.



**Fig. 3.** The frequencies of territorial behavior in the field test in 2008. (A) Long-call. (B) Long-chase. \*:  $P < 0.05$ .

the first 3 min after being placed in the equipment, allowing them time to adapt to the new equipment. We recorded the total time of attacking and total time of approaching the female for 6 min. If the attacking time was greater than 30 s within the first three minutes, the experiment was stopped to avoid severe injuries to animals.

2.4. Statistical analyses

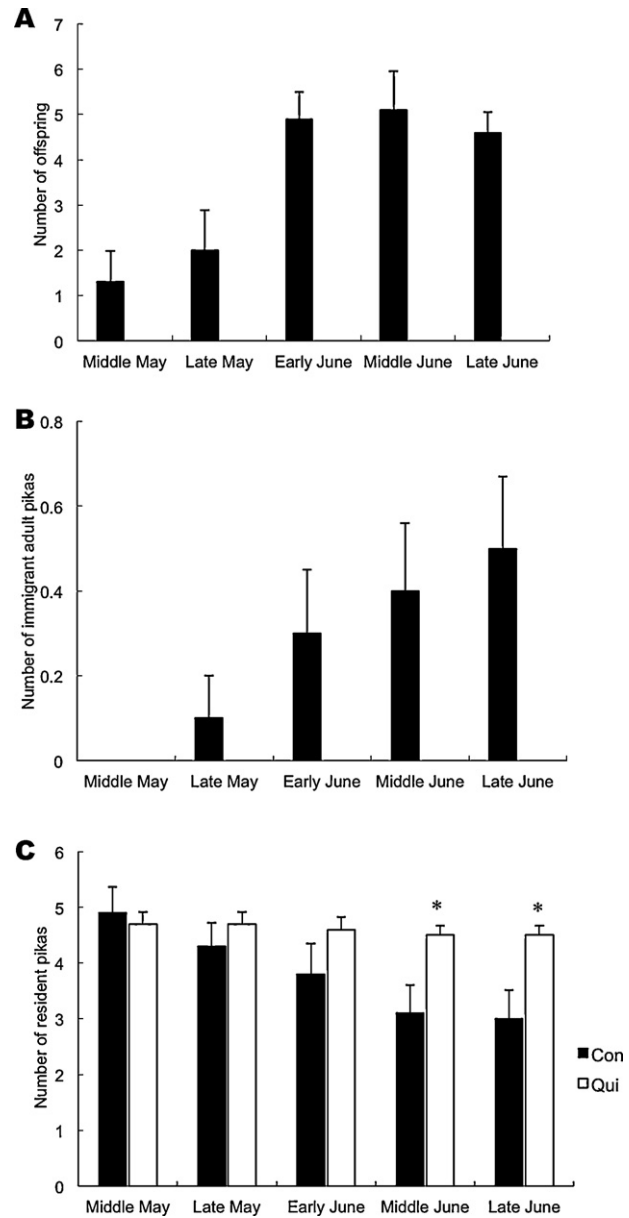
A general linear model (GLM) was used to analyze differences in the frequency of long-call and long-chase, number of resident pikas, newborn pikas and immigrant pikas between the control and quinestrol-treated group in 2008; the density of resident pikas was the co-variable. The significance of the frequency of long-calls and long-chases, number of resident pikas, newborn pikas, immigrant pikas and the frequency of aggressive or mating behavior in 2009 of control and experimental groups were analyzed using the Mann–Whitney *U* test after the Kruskal–Wallis test. The significance level was set at 0.05. Spearman correlations were used to detect the correlations between the number of newborn pikas per family group and the frequency of long-calls and long-chases. All analyses were conducted using SPSS version 17 (SPSS Inc., Chicago, USA).

3. Results

3.1. Field test

3.1.1. Territorial behavior

The frequency of long-calls in the quinestrol-treated area was significantly higher than those in the control area in mid-May, late May, early June and mid-June in 2008 (Fig. 3A). The frequency of long-chases in the quinestrol-treated group was significantly



**Fig. 4.** The structure of family groups in the field test in 2008. (A) The mean number of newborn pikas. (B) The mean number of immigrant pikas. (C) The mean number of resident adult pikas. \*:  $P < 0.05$ .

higher than those in the control group in late May, early June and mid-June in 2008 (Fig. 3B). The territorial behaviors of both groups steadily decreased with time and the differences became insignificant by late June when breeding of pikas ceased.

3.1.2. Family structure

The mean number of resident adults in the control area decreased from approximately five to four per family in 2008, while the quinestrol-treated area showed no change. The mean number of newborn pikas per family increased from mid-May to mid-June in the control area (to more than four until mid-June), while in the quinestrol-treated area there was no recruitment of newborn pikas into families (Fig. 4A). There was also an obvious increase in the mean number of immigrant adults in the control area: it reached approximately 0.5 pikas per family group by late June, while no immigrant adult was observed in the quinestrol-treated families

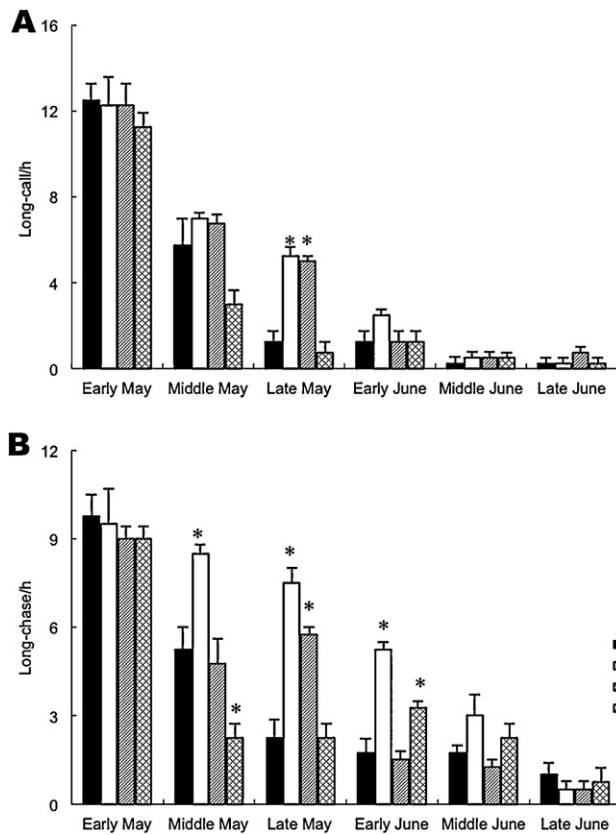


Fig. 5. Frequency of territorial behaviors in the enclosure test in 2009. (A) Long-call. (B) Long-chase. \*:  $P < 0.05$ .

(Fig. 4B). The differences of resident pikas between the two areas became significant by mid- to late June (Fig. 4C).

### 3.2. Enclosure test

#### 3.2.1. Territorial behavior

The frequency of long-calls in the Qui group was significantly higher than those of the control group in late May (Fig. 5A and Table 1), while the frequency of long-chase in the Qui group was higher than those of the control group in mid-May, late May and early June (Fig. 5B and Table 1). These results are very similar to those observed in the field experiment. The frequency of both long-calls and long-chases in the C-R group was significantly higher than those of the control group in late May. The frequency of long-chases in the Q-I group was significantly lower than those of the control group in mid-May. However, the frequency of long-chases became higher than the control group by the early June due to removal of newborn pikas (Fig. 5 and Table 1). Table 1 shows the results in May, indicating that presence of newborn pikas can significantly reduce the frequencies of territory behaviors.

The frequency of both long-calls (Spearman correlation =  $-0.407$ ,  $P = 0.048$ ) and long-chases (Spearman correlation =  $-0.592$ ,  $P = 0.002$ ) were significantly and negatively correlated with the number of newborn pikas per family in all four enclosures (Fig. 6).

#### 3.2.2. Family structure

The number of newborn pikas steadily increased from approximately two per family group in May to four young pikas per family by mid-June in the control groups (Fig. 7A). In the Qui group, in which all male pikas were infertile, there were almost no

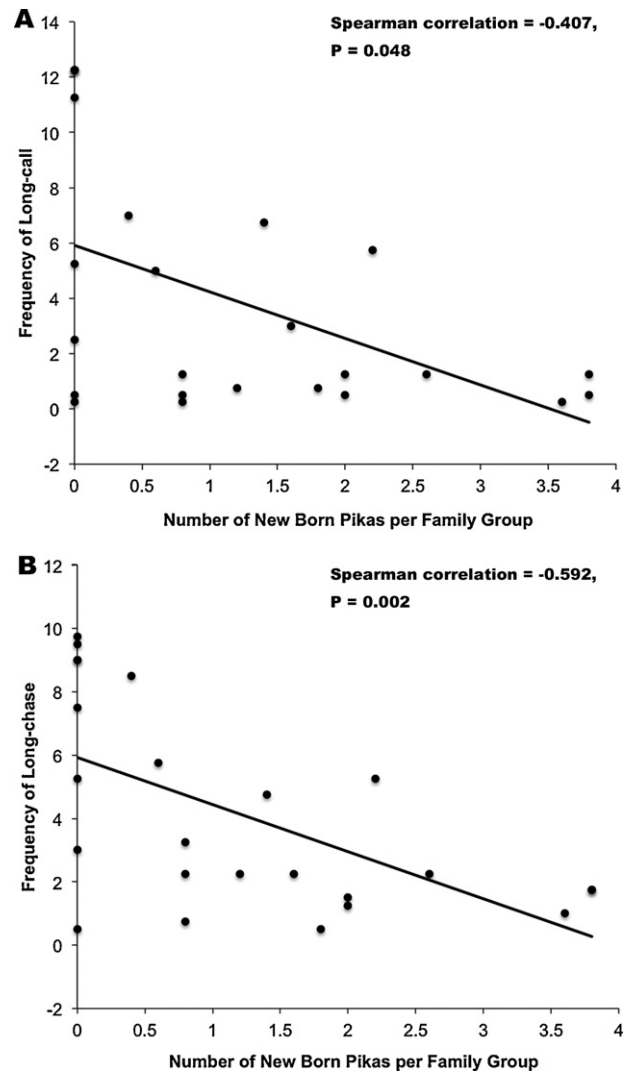
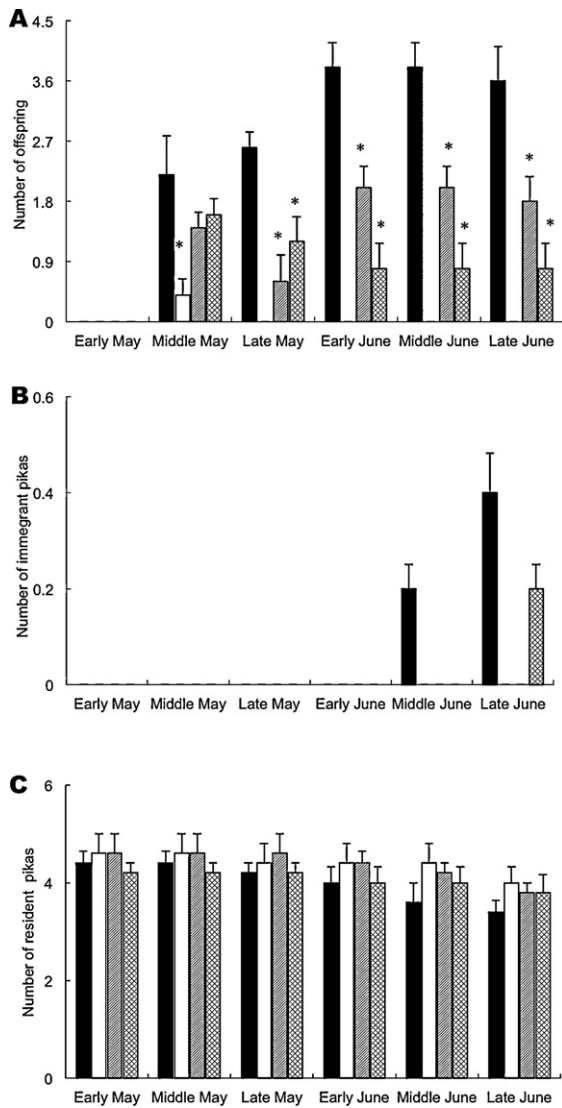


Fig. 6. Mean number of the newborn pikas per family group and the frequency of long-call and long-chase in Experiment II. (A) Long-call. (B) Long-chase.

newborn pikas during the breeding season except a few in mid-May. In the Q-I group, the number of newborn pikas per family group steadily decreased because males were sterilized. In the C-R group, the number of newborn pikas was low due to artificial removal, but increased again when normal females bred. The number of newborn pikas per family in the C-R and Q-I groups was between that of the control and Qui groups.

There were 0.2 and 0.4 immigrant adult pikas per family group observed in mid-June and in late June, respectively, in the control group (Fig. 7B). In the Q-I group, there were 0.2 immigrant pikas per family in late June. There were no immigrant adult pikas in any families in the Qui and C-R groups.

The number of resident adult pikas in the control and Qui groups was similar to those in the Experiment I (Fig. 7C). Resident pika numbers gradually decreased from more than four to less than four pikas per family group in the control group during May and June. The Qui group did not experience a decline in resident pikas during June and July in 2009. In mid- and late June, the Qui group exhibited a higher number of resident adult pikas than controls, however this difference was not significant. In C-R and Q-I groups, the number of resident adult pikas was between those of the control and Qui groups in June.



**Fig. 7.** The structure of family groups in the enclosure test in 2009. (A) Mean number of newborn pikas. (B) Mean number of immigrant pikas. (C) Mean number of original adult pikas.

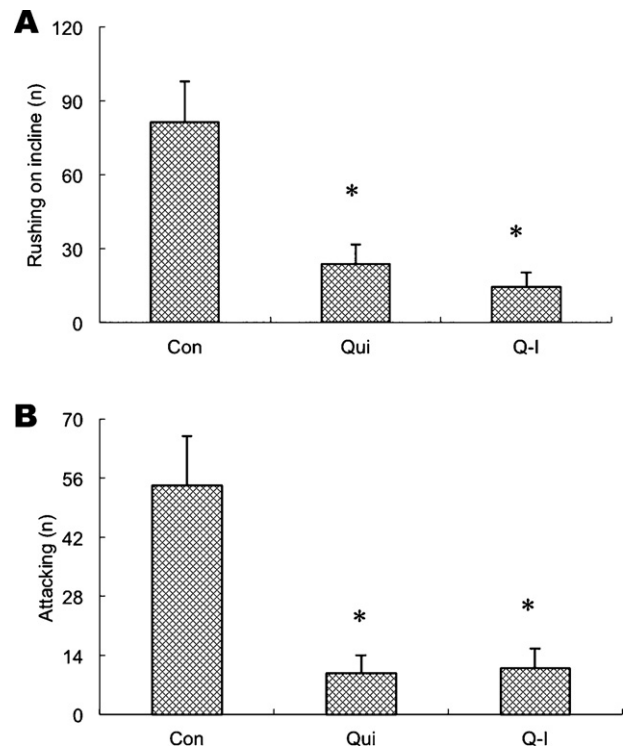
3.2.3. Aggressive and mating behaviors

The cage test indicated that the frequency of rushing towards mates was higher in the control group than the Q-I and Qui groups (Fig. 8A). The frequency of aggressive behavior was also significantly higher in the control group compared to the Q-I and Qui groups (Fig. 8B).

4. Discussion

Quinestrol is observed to be an effective sterilization agent for males of many rodent species according to laboratory and field tests (Huo et al., 2007; Liang et al., 2006; Wan et al., 2006; J. Zhang et al., 2004; Z. Zhang et al., 2004; Zhang et al., 2005, 2006; Zhao et al., 2007). Our field test indicated that quinestrol can effectively sterilize male plateau pikas in the Qinghai-Tibetan plateau, and a single baiting in early May can cause breeding failure of females throughout the breeding season under natural conditions (Liu et al., in press).

The mechanism of male sterilants in the fertility control of rodent population has long been discussed (Kirkpatrick and Turner, 1985; Gao and Short, 1993). For animals with mating systems that involve promiscuity, males have a high chance to get access



**Fig. 8.** Frequency of aggressive and mating behavior of pikas in control and Qui groups in Experiment II. (A) Frequency of approaching females along the slope plate (mating behavior). (B) Frequency of attacking the other male (aggressive behavior). \*:  $P < 0.05$ .

to fertile females, thus it is hard to achieve efficacy with male sterilization unless a very high proportion of the male population are sterilized. In monogamous or polygamous mating systems, male sterilization can achieve better results than traditional culling because infertile males can defend their mating partners (Zhang, 2000). Several previous studies have suggested that the mating system of plateau pikas may be polygamy or promiscuity or their mixture (Dobson et al., 1998, 2000; Qu et al., 2008), so male fertility control would not be predicted to yield good results (Zhang, 2000). Our study revealed that in the quinestrol-treated group, sterilized males showed enhanced territory behaviors, preventing invasion of adult pikas from other families into their own families. The high efficacy of male sterilant in controlling fertility of female pikas indicate that the mating system of pikas should be largely polygamy under the high frequency of territorial behavior.

Prior to our study, the behavioral mechanisms of male sterilization had not been investigated in both laboratory and field conditions simultaneously. Our study exhibits a novel underlying mechanism for how male sterilization limits reproduction and the recruitment of newborn animals in plateau pikas during the breeding season. Male pikas treated with quinestrol showed significantly decreased testosterone levels (Liu et al., in press), but significantly higher frequency of territorial behavior compared to controls this study.

Aggressive or territorial behavior is often positively related to levels of testosterone (Bahrke et al., 1996; Brain and Haug, 1992; Edwards, 1969; Ogawa et al., 1996; Rowe and Swanson, 1977). In male hamsters, castration is shown to reduce aggression and social dominance, while testosterone replacement can restore these capacities (Zhang et al., 2001a, 2001b). Estrogens, like quinestrol, can decrease production of testosterone. Estrogen is a direct suppressor of androgen production in the hypothalamo-pituitary-gonadal axis (Hayes et al., 2000), for in

target cells, plenty of aromatase exists to convert testosterone to estradiol (Bagatell et al., 1994). Androgens, which mainly exist in the form of testosterone in mammals, are essential for maintaining secondary sex characteristics and aggressive and sexual behavior in males (Wilson, 1999; Zuloaga et al., 2008; Erpino and Chappelle, 1971). Theoretically, quinestrol should reduce the aggressive or territorial behavior by decreasing androgen production.

However, aggressive behaviors are not always determined by hormone levels. For example, in non-breeding seasons aggression in male hamsters does not decrease (Zhang et al., 2001a, 2001b). Aggression of hamster species such as golden hamsters, *Mesocricetus auratus* and Siberian hamsters, *Phodopus sungorus* is promoted by short-day conditions (Garrett and Campbell, 1980; Jasnow et al., 2000). Aggressive behavior can also be retained in male adults as a learned behavior even if testosterone is low. In our study, we found the territory behaviors of sterilized male pikas became higher than non-sterilized males in both field and enclosure conditions.

Experiment II was used to explore other factors (i.e. presence of newborn pikas) for the increase of territorial behavior while the testosterone level decreased in sterilized male pikas. We found that the presence of newborn pikas was the predominant factor determining their territorial behavior. In the Qui group without newborn pikas, male sterile pikas showed consistently higher levels of territorial behavior than those of the control group throughout the breeding season. Both groups showed steady decreases in territorial behavior by the end of breeding, suggesting these behaviors are related to the breeding performance of pikas. In the C-R and Q-I groups the frequency of territorial behavior increased when the number of newborn pikas decreased (by removal or by sterilization), but decreased once the number of newborn pikas increased again (by females reproducing again after removal or by introduction of pregnant females). The levels of long-calls and long-chases were negatively related to the number of newborn pikas in the family. It is notable that although the territorial behavior exhibited an elevation in the field or enclosure, the aggressive behavior of individual showed a significant decrease in a cage condition (Fig. 8), suggesting that increase of territorial behavior is much determined by social structure of plateau pikas, not only the testosterone level. Thus, social stress, but not the physiological state of the individuals, might be the main force to support higher territorial behavior in plateau pikas.

A new question emerging from this study is why the number of newborn pikas affects the performance of territorial behavior in adult pikas? There are two potential explanations. First, male pikas in the quinestrol group have to increase their territorial behavior to protect their females from mating with intruding males. The appearance of newborn pikas may be the signal that females have produced their own offspring. This may be similar to sperm competition theory in which males will continue to protect mated females to ensure their genes will be successfully carried by the female (Lifjeld et al., 1994; Jivoff, 1997). Second, male pikas in the control group have to reduce aggression levels to reduce potential harm to newborn pikas (Soltis et al., 2000; Packer, 1980). The first hypothesis is supported by the observation that immigration of another family in the Qui group was mostly prohibited.

Plateau pikas can re-organize their families by recruiting newborn pikas from other families (Wang and Smith, 1989; Smith and Wang, 1991; Dobson et al., 2000). Exchange of family members would benefit gene flow and increase genetic diversity in pika populations (Yin et al., 2009; Peacock, 1997). This observation is supported by our results from the control groups in both enclosure and field conditions. We observed that few adult pikas invaded successfully into other families. In the Qui group, there was no immigration among families, which may benefit long-term population control but reduce genetic diversity of the population. This issue needs to be investigated further.

## 5. Conclusion

Our results reveal that quinestrol significantly increased territorial behavior in male plateau pikas, but reduces their aggression and mating behavior. Territorial behavior in plateau pikas is negatively related to the number of newborn pikas. Single-baiting of quinestrol effectively reduced the pregnancy rate of female pikas by preventing invasion of adult male pikas from other families, probably due to increases in the territorial behavior of pikas. Our study provides a good example of how male sterilization can achieve successful fertility control through behavioral modification.

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