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Autocidal control of ticks by silencing of a single gene by RNA interference

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Abstract

Ticks impact human and animal health worldwide and new control methods are needed to circumvent drawbacks of tick control by acaricide application including selection of drug resistant ticks and environmental pollution. Using RNA interference we silenced the expression of a single gene, subolesin, and produced ticks with diminished reproductive performance and prevented successful mating and production of viable offspring. We propose a sterile acarine technique (SAT) for reduction of tick populations by release of subolesin-silenced ticks. Conservation of subolesin among tick species suggests that SAT may be useful for control of many medically and economically important tick species.

Keywords: 4D8; RNAi; Tick; Vaccine; Subolesin; Autocidal control; Sterile

Ticks are obligate hematophagous ectoparasites of wild and domestic animals and humans, and are distributed worldwide from Arctic to tropical regions. These ectoparasites are classified in the subclass Acari, order Parasitiformes, suborder Ixodida [1]. Some tick species, such as *Boophilus microplus* (Canestrini), complete their entire life cycle on a single host, while others, such as *Dermacentor* spp., utilize separate hosts for larvae, nymphs, and adults. Metastriate ticks, such as *Dermacentor* spp., mate only while feeding on the host [2].

Ticks are important vectors of pathogens and the resulting diseases greatly impact human and animal health and food production worldwide [3,4]. For example, *Dermacentor andersoni* Stiles and *Dermacentor variabilis* (Say) vector a rickettsial pathogen, *Anaplasma marginale*, that causes the major tick-borne disease of cattle in the United States, anaplasmosis [5].

Control of ticks has been attempted primarily by application of acaricides, a method accompanied by dual drawbacks of environmental contamination and by selection of pesticide-resistant ticks [6,7]. Vaccines administered to host animals have shown promise as a tick control method, but their use and efficacy have been limited [6,7].

Novel methods of tick control, similar to sterile insect technique (SIT) developed for control of insect pests, could circumvent the drawbacks of acaricides [8,9]. Release of large numbers of laboratory-sterilized insects has been practice for several years [8,9]. Genetically based methods for control of agriculturally and medically important insects (e.g., boll weevils, screw worms, bollworms, fruit flies, tsetse flies, and mosquitoes) have been developed [9]. For example, genetic manipulation of a transgene combination, as proposed by Gong et al. [10], may improve SIT against medflies and replace control methods based upon irradiation sterilization. An added advantage of genetic

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manipulation in this case is that a dominant lethal gene in the sterile flies can also serve as a genetic marker.

We identified the gene subolesin by expression library immunization in a mouse model of larval tick infestations [11–14]. The subolesin gene was shown to be highly conserved among tick species, and when the expressed protein was used as a vaccine antigen, hosts were protected against tick infestations [11–14]. RNA interference (RNAi) silencing of subolesin expression profoundly affected gut and reproductive tissues in male and female ticks; females were unable to feed productively as evidenced by their failure to reach repletion and to oviposit normally [15,16]. Although the biological function of subolesin is currently unknown, the silencing of this gene by RNAi resulted in pleiotropic effects on tick organs and affected various physiological processes, including the modulation of tick blood ingestion and reproduction. These data have therefore suggested a highly specialized and conserved biological function for subolesin [16].

Herein, we describe a potential autocidal control method for *D. variabilis* that is based upon RNAi silencing of the expression of the subolesin gene. Specifically, silencing of this gene disrupted reproductive development in both female and male ticks, and their attempts to mate were unproductive. Female ticks did not become replete or produce viable eggs, and male ticks did not produce mature spermatophores. Control by release of male ticks in which silencing of subolesin has been effected may have application for reducing populations of many tick species. Release of massively produced sterile male and female ticks reared via artificial feeding systems could become a sterile acarine technique (SAT) suitable for use alone or in combination with other control methods, such as vaccination and focused use of acaricides.

Materials and methods

Ticks. Adult ticks were obtained from the laboratory colony maintained at the Oklahoma State University Tick Rearing Facility. Sheep were housed at the Tick Rearing Laboratory with the approval and supervision of the OSU Institutional Animal Care and Use Committee. Off-host ticks were maintained in a 12 h light:12 h dark photoperiod at 22–25 °C and 95% relative humidity.

Generation of dsRNA. Oligonucleotide primers containing T7 promoter sequences for in vitro transcription and synthesis of dsRNA (382 bp; full length cDNA, 498 bp [16]) were synthesized to amplify DNA encoding *D. variabilis* subolesin using oligonucleotide primers D8AAT75: 5′-TAATACGACTCACTATAGGGTACTGACTGGGATCCCCTGCACAGT-3′ and D8DVT73: 5′-TAATACGACTCACTATAGGGTACTCGAGCTTGGTGGAAAGGACG-3′. RT-PCR and dsRNA synthesis reactions were performed as previously described [15,16], using the Access RT-PCR system (Promega) and the Megascript RNAi kit (Ambion, Austin, TX, USA). The dsRNAwas purified and quantified by spectrometry.

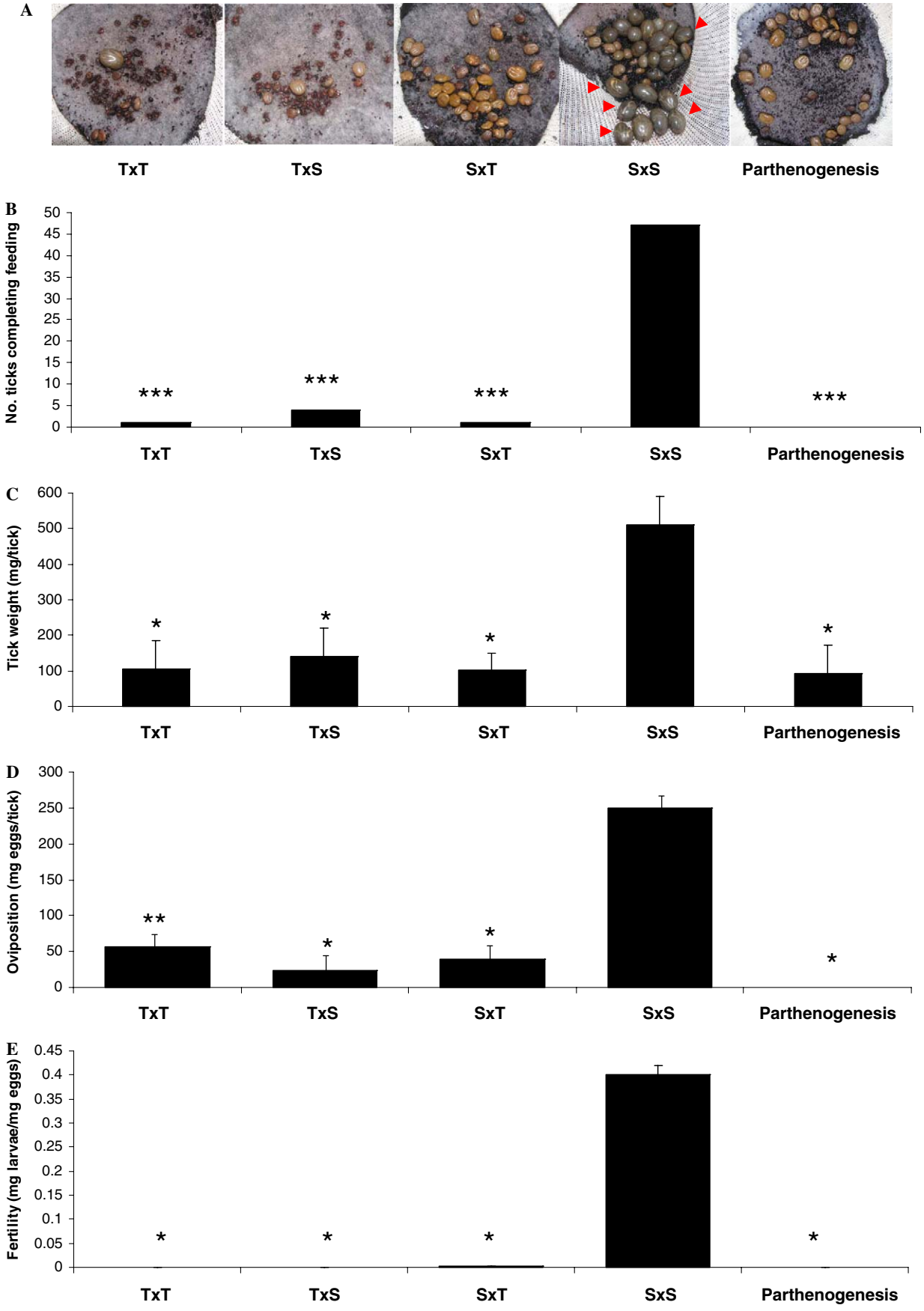
Injection of ticks with dsRNA. Dermacentor variabilis ticks were injected with approximately 0.2–0.5 μl dsRNA ($6\times10^{10}-2\times10^{11}$ molecules per/μl) in the lower right quadrant of the ventral surface of the exoskeleton of ticks [15,16]. The injections were done with a Hamilton syringe with a 1 in., 33 gauge needle.Control ticks were injected with injection buffer (10 mM Tris–HCl, pH 7, 1 mM EDTA) or were left uninjected. The ticks were held in a humidity chamber for 1 day after injection and then they were allowed to feed on a sheep. Female ticks that fed to repletion or those that were removed from the sheep after 10 days of feeding were collected, counted, weighed, and evaluated for oviposition by weighting the egg mass oviposited by each tick. Egg fertility was evaluated by weighting the larvae obtained from each egg mass.

Analysis to confirm subolesin gene silencing by RT-PCR. Salivary glands and guts were dissected from groups of five *D. variabilis* ticks from mock-injected and dsRNA-injected groups after feeding. Total RNA was isolated and analyzed for subolesin transcripts by RT-PCR as described above using oligonucleotide primers RA4D85:ATGGCTTGTGCGACATTAAAGCGGAC and RA4D83:TTACGACAAATAGCTGGGCGTAGC. The tick 16S rRNA (approx. 215 bp) was analyzed as a control unrelated target sequence using oligonucleotide primers T16S5 (5′-GACAAGAAGACCCTA-3′) and T16S3 (5′-ATCCAACATCGAGGT-3′). Control reactions were performed using the same procedures but without RT to test for DNA contamination in the RNA preparations and without RNA added to detect contamination of the PCR. PCR products were electrophoresed on 1% agarose gels to check the size of amplified fragments by comparison to a DNA molecular weight marker (1 kb DNA Ladder, Promega).

Data analysis. The analysis was done with female ticks. The weight of ticks after feeding, oviposition, and fertility was compared by one-tailed Student's t test for samples with unequal variance ($P=0.01$) between control ticks injected with buffer alone (S × S) and groups containing subolesin dsRNA-injected ticks (T × T, T × S, and S × T) or parthenogenic ticks. Tick mortality and tick engorgement (number of ticks completing feeding) were evaluated as the ratio of dead and engorged ticks after 10 days of feeding, respectively, to the number of ticks used for infestation. Tick mortality and engorgement were compared between dsRNA and mock-injected ticks by χ^2 test ($\alpha=0.01$). In the third series of experiments to evaluate if pairing of untreated female ticks with subolesin dsRNA-injected males would render females unable to mate with normal males Cells 1–4 were compared between them by Analysis of Variance (ANOVA) followed by a series of Tukey's post hoc tests for pair comparisons ($\alpha=0.05$).

Video recording of tick pairing. A harness was custom-made for the sheep that was constructed from canvas, a molded Styrofoam cushion, and Velcro closures. The harness facilitated the positioning of two lights and a video camera over the tick feeding stockinette that was glued to the back of the sheep. A KPC-S38CZ mini security video recorder (www.cu1.com) was used to record tick mating behavior and the video was computerized using Windows XP operating system and a ProTech Videocard and DiViS software (www.securitycameraworld.com). The video camera was mounted in the center of the apparatus for a direct overhead view of the ticks' feeding cell, using a light source of two LED cool lights (http://www.redhillgeneralstore.com/A13791.htm). Both lights were mounted laterally on each side of the camera to provide optimum lighting without shadowing. Ticks were placed in the stockinette 48 h prior to

Fig. 1. Silencing of the subolesin gene expression disrupted reproductive development in female and male ticks, and attempts to mate were unproductive. *D. variabilis* females and males were injected with subolesin dsRNA (T) or injection buffer alone (S), and all possible pairing combinations were made between them in separate feeding cells on a sheep. A group of untreated female ticks were fed without males to evaluate parthenogenesis in this species. (A) Fully engorged female ticks (exemplified with red arrows) were obtained in the control S × S group only. Parthenogenic ticks and female or male ticks in which subolesin gene expression were silenced by RNAi resulted in a significant reduction in the number of female ticks that completed feeding (B), tick weights (C), oviposition (D), and egg fertility (E) as compared with untreated SxS controls. $^{*}P<0.001$; $^{**}P<0.01$ (one-tailed Student's t test for samples with unequal variance); $^{***}\alpha<0.01$ (χ^2 test).


A
TxT
TxS
SxT
SxS
Parthenogenesis
B
No. ticks completing feeding
50
45
40
35
30
25
20
15
10
5
0
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TxT
TxS
SxT
SxS
Parthenogenesis
C
Tick weight (mg/tick)
600
500
400
300
200
100
0
*
*
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TxS
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SxS
Parthenogenesis
D
Oviposition (mg eggs/tick)
300
250
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TxT
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Parthenogenesis
E
Fertility (mg larvae/mg eggs)
0.45
0.4
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SxS
Parthenogenesis

video recording. The subolesin dsRNA-injected male ticks were painted red, while the saline-injected males were painted white using Boyd spray enamel (Testor Corporation, Rockford, Ill). For spray painting, ticks were placed ventral side down on double sticky tape and their mouth parts were covered with tape to avoid paint accumulation. The pairing of the ticks was recorded daily by observation and by digital photography. Video recording of tick pairing was ongoing for 7 days.

Results and discussion

In an effort to devise new methods for tick control, we investigated the possibility of using RNAi to develop a SAT method for ticks, similar to the SIT commonly used successfully to suppress populations of economically important insect pests. Release of hybrid sterile ticks to mate with wild females that then produce unfit hybrid offspring was advanced as a putative alternative control method explored for the one-host *Boophilus* spp [8,17,18]. Although hybrid sterility can be considered a subset of the sterile release approach to population suppression, pre-mating isolation factors between the target tick species and/or hybrids appear to limit the utility of this method [18].

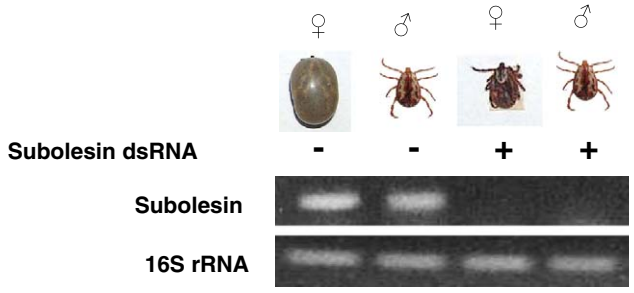

Fig. 2. Silencing of subolesin expression by RNAi in ticks. The expression of subolesin mRNA was analyzed by RT-PCR in female and male ticks injected with subolesin dsRNA (+) or with injection buffer alone (−) and compared with the expression of tick 16S rRNA.

Recently, RNAi provided a means for genetic manipulation of ticks, as well as an efficient way to characterize gene function in these organisms [15,16,19]. We used RNAi to silence the gene subolesin (previously called 4D8) whose expressed protein was found to be protective against tick infestations when used as a vaccine antigen. This highly conserved gene encodes for a protein that was protective against all tick developmental stages when tested in both cDNA and recombinant protein immunization trials [11–14].

RNAi silencing of the subolesin gene disrupted reproductive tissue development in female and male ticks, and attempts by the injected ticks to mate were unproductive. In the first series of experiments, *D. variabilis* (American dog tick) females and males were injected with subolesin dsRNA (T) or with injection buffer alone (S), and all possible pairing combinations were made between principal and control ticks employing separate feeding cells attached to a sheep. A group of untreated female ticks

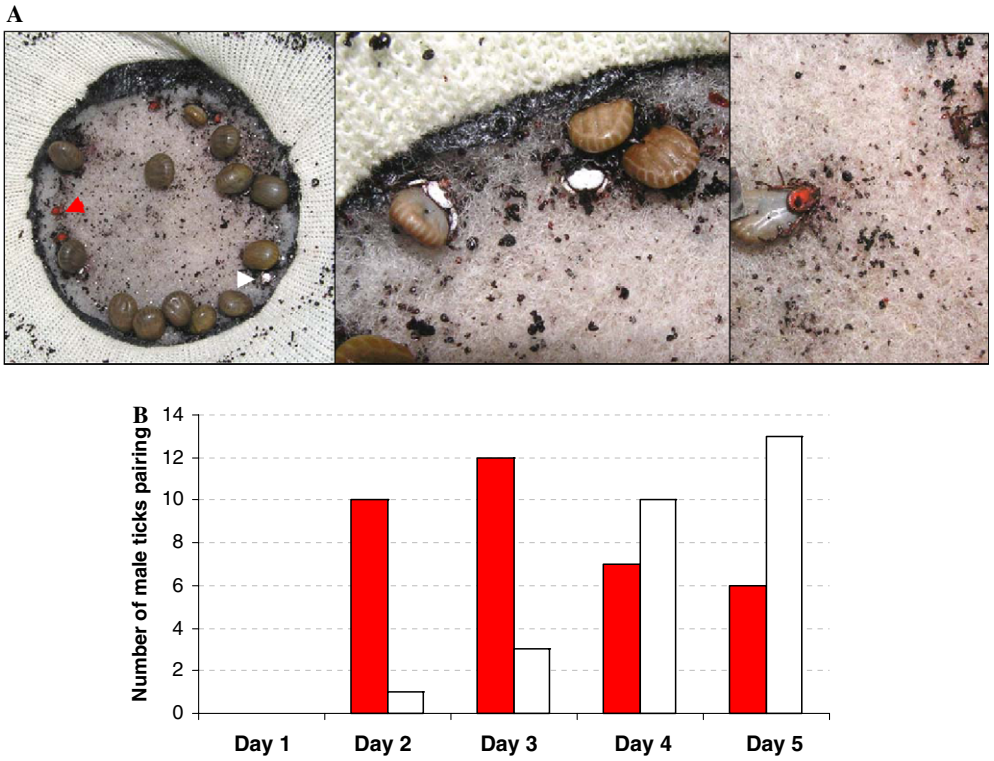

Fig. 3. Subolesin dsRNA-injected male pair with female ticks. (A) Untreated females ($N = 20$) were used together with males injected with subolesin dsRNA ($N = 20$) and control saline-injected males ($N = 20$) marked with red and white paint, respectively (arrows on left-hand image). Middle and right-hand images are enlargements of the left-hand image. (B) After infestation, pairing of dsRNA-injected (red bars) and control (white bars) male ticks was observed and recorded daily by video recording and visual inspection.

was fed in the absence of males to evaluate the possibility of parthenogenesis in this tick species (Fig. 1). Silencing of subolesin expression was confirmed by RT-PCR on ticks examined after completion of feeding (Fig. 2). Silencing of subolesin gene expression by RNAi in male or in female ticks resulted in a significant reduction in the number of female ticks that completed feeding in all pairing combinations when compared to the S × S controls (Figs. 1A and B). The principals had reduced weights (Figs. 1A and C), oviposition was impaired (Fig. 1D), and eggs that were produced did not hatch into larvae (Fig. 1E) as compared with untreated controls that reproduced normally. Female tick mortality was higher ($\alpha < 0.01$; χ^2 test) in the T × T (78%) and T × S (68%) groups including subolesin dsRNA-injected females, as compared with the S × T (6%), S × S (6%), and female only (8%) groups.

As shown in previous subolesin RNAi experiments with other tick species [15,16], these data demonstrated that silencing of the subolesin gene expression interfered with female feeding and oviposition, and caused reduced fertility in males as well. Microscopic examination of guts, salivary glands, and reproductive tissues confirmed the pleiotropic effect of subolesin silencing on these organs [16].

We believe that silencing of subolesin expression by RNAi may provide a new means of controlling ticks. To test this hypothesis, a second series of experiments was done to evaluate *D. variabilis* infestations in feeding cells on a sheep of ticks injected with subolesin dsRNA or control ticks injected with buffer alone. The weight of female ticks was determined for replete ticks that dropped from the host and others removed after ten days of feeding after the control ticks had become replete. In the first experiment, 20 males and 10 females for each treatment (buffer- or dsRNA-injected) were placed together in a feeding cell. After 10 days, 30% of the females were replete (tick weight $\geqslant$ 300 mg), 30% were partially engorged (100 mg $\leqslant$ tick weight $<$ 300 mg), 15% had failed to engorge (tick weight $<$ 100 mg) and five ticks had died. The distribution of weights among females suggested that crosses between ticks injected with subolesin dsRNA and control ticks may have occurred. In the second experiment, 20 untreated females were placed in a feeding cell with 20 males injected with subolesin dsRNA and 20 control (buffer-injected) males marked with red and white paint, respectively (Fig. 3). Male/female pairings were observed and documented daily by video recording and visual inspection. We expected that female ticks would pair equally with dsRNA-injected and control

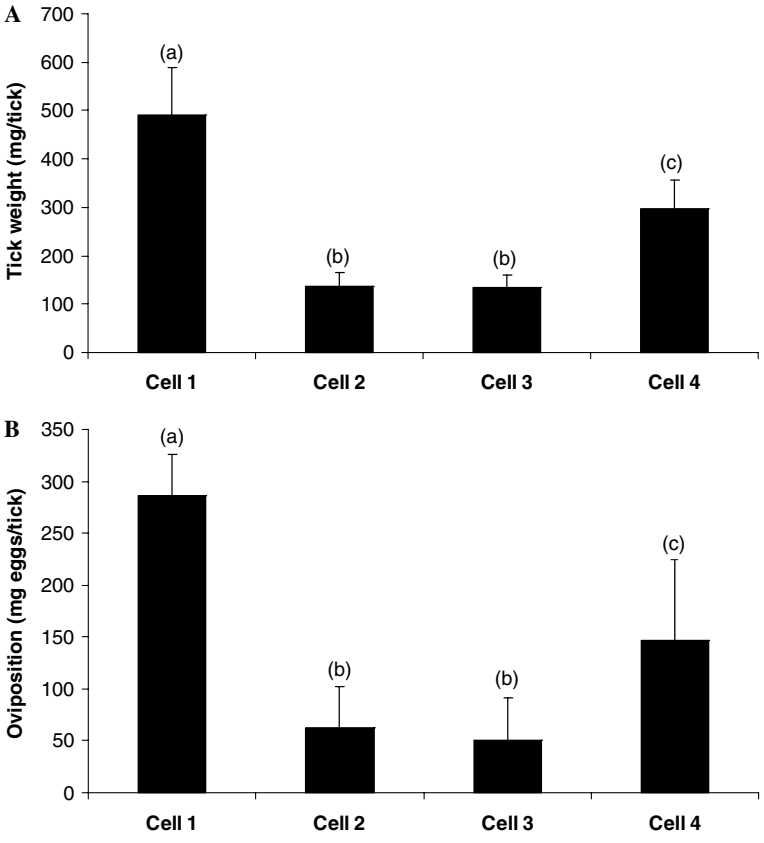

Fig. 4. Pairing of untreated female ticks with subolesin dsRNA-injected males rendered females unable to mate with normal males. Four cells were used to infest a sheep with *D. variabilis* ticks. Cell 1, 20 untreated females and 20 saline-injected males; Cell 2, 20 untreated females and 20 subolesin dsRNA-injected males; Cell 3, same as Cell 2, but at the same time as the Cell 1 females began to replete (7 days post-infestation), the subolesin dsRNA-injected males were removed and replaced with 20 untreated males; and Cell 4, 20 untreated females that were allowed to feed for 7 days, after which untreated males were added. The weight of females (A) and oviposition (B) was evaluated and compared between groups. Different letters denote statistical significant differences ($\alpha < 0.05$; Analysis of Variance (ANOVA) followed by a series of Tukey's post hoc tests for pair comparisons).

males. However, inexplicably, while the female ticks paired with both principal and control males (Figs. 3A and B), they paired first with dsRNA injected males and later with the buffer-injected controls (Fig. 3B). The timing of repletion of the female ticks and of oviposition (both evidence of successful mating) corresponded with the interval from female/control male pairings rather than with the earlier, apparently unsuccessful, mating with the treated males. The weight of female ticks fell into two different groups ($P < 0.0005$) that probably reflected mating with dsRNA and buffer-injected males. Six female ticks, which completed feeding on days 5 and 6 after feeding, were smaller (average weight $\pm$ SD, 242 ± 79 mg), while ticks that fed to repletion on the 7th day were fully engorged (511 ± 55 mg).

For efficient control, female ticks that had mated with subolesin dsRNA-injected males would need to be refractory to subsequent mating with untreated males. Therefore, in a third series of experiments we tested the hypothesis that pairing of untreated female ticks with subolesin dsRNA-injected males would render them refractory to mating with normal males. Four cells were used on a sheep infested with *D. variabilis* ticks in the following groups: Cell 1, 20 untreated females and 20 buffer-injected males; Cell 2, 20 untreated females and 20 subolesin dsRNA-injected males; Cell 3, same as Cell 2, but at 7 days post-infestation when females in Cell 1 began to drop off the host, the subolesin dsRNA-injected males were removed and replaced with 20 buffer-injected males; and Cell 4, 20 untreated females were allowed to feed for 7 days in the absence of males, after which twenty untreated males were added. Cell 4 served as a control for Cell 3 by determining whether females would successfully mate with the males after the 7-day delay. Females from all groups were weighed when replete or when removed from the host, and oviposition was evaluated. The results of this experiment demonstrated that although female ticks paired with subolesin dsRNA-injected males (Cells 2 and 3), they were not successfully mated because they failed to feed to repletion or oviposit; even when untreated males were introduced into Cell 3 at 7 days reproductive failure still occurred (Fig. 4A). That is females were refractory to mating with normal males if first exposed to dsRNA-injected ones. Although mating of the females in Cell 4 was artificially delayed, the number of females that fed to repletion and oviposited was comparable to that of the controls in Cell 1; adverse effects seemed to be limited to lower weight gains in these females (Figs. 4A and B). Oviposition of female ticks paired with subolesin dsRNA-injected males in Cells 2 and 3 was reduced by approximately 80% compared to control female ticks in Cell 1 and 60% to those in Cell 4 (Fig. 4B). The eggs from ticks in Cells 1 and 4 were fertile and hatched into viable larvae, but the few eggs produced by females in Cells 2 and 3 failed to hatch. The inhibition of re-mating after successful mating is common in Ixodidae tick species [20,21]. The results reported herein suggested that subolesin dsRNA-injected males attempted to successfully mate with female ticks. However, although they failed to fertilize the females, the males apparently rendered the females refractory to a subsequent mating.

Collectively, these experiments demonstrated the feasibility of control of ticks by the RNAi silencing of a single gene. Silencing of this gene resulted in greatly reduced fertility of both female and male ticks and complete reproductive failure in some ticks. This approach to control would involve transient modification in gene expression that could be applied to many tick species without the need to produce transgenic organisms. Classical approaches for genetic control of ticks such as mutagen-induced and hybrid sterility have had limited applicability due to technical difficulties in inducing sterility of male and female ticks and behavioral factors associated with sterile hybrids [9,18,22] that were not observed in the experiments described herein.

Practical use of this gene silencing RNAi method would require massive production of sterile male and female ticks for release, and such production may be possible. The recent demonstration of silencing gene expression through capillary feeding (CF) of specific dsRNAs in ticks [23], combined with the application of CF to other tick species [24], provides a basis for design of methods to mass produce RNAi-treated ticks. This control method for ticks could prove to be effective when used alone or in combination with other methods such as vaccination and application of specifically targeted acaricides.

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References

[1] S.C. Barker, A. Murrell, Systematics and evolution of ticks with a list of valid genus and species names, Parasitology 129 (2004) S15–S36.
[2] Y.S. Balashov, Nutrition and course of spermatogenesis in male ixodids, Dokl. Akad. Nauk. USSR 110 (1956) 1133–1136.
[3] A. Estrada-Peña, F. Jongejan, Ticks feeding on humans: a review of records on human-biting Ixodoidea with special reference to pathogen transmission, Exp. Appl. Acarol. 23 (1999) 685–715.
[4] P. Parola, D. Raoult, Tick-borne bacterial diseases emerging in Europe, Clin. Microbiol. Infect. 7 (2001) 80–83.
[5] K. Kocan, J. de la Fuente, E.F. Blouin, J.C. Garcia-Garcia, *Anaplasma marginale* (Rickettsiales: Anaplasmataceae): recent advances in defining host-pathogen adaptations of a tick-borne rickettsia, Parasitology 129 (2004) S285–S300.
[6] J. de la Fuente, K.M. Kocan, Advances in the identification and characterization of protective antigens for development of

recombinant vaccines against tick infestations, Expert Rev. Vaccines 2 (2003) 583–593.
[7] P. Willadsen, Anti-tick vaccines, Parasitology 129 (2004) S1–S21.
[8] W.N. Beesley, The ecological basis of parasite control: ticks and flies, Vet. Parasitol. 11 (1982) 99–106.
[9] F. Gould, P. Schliekelman, Population genetics of autocidal control and strain replacement, Annu. Rev. Entomol. 49 (2004) 193–217.
[10] P. Gong, M.J. Epton, G. Fu, S. Scaife, A. Hiscox, K.C. Condon, G.C. Condon, N.I. Morrison, D.W. Kelly, T. Dafa'alla, P.G. Coleman, L. Alphey, A dominant lethal genetic system for autocidal control of the Mediterranean fruitfly, Nat. Biotecnol. 23 (2005) 453–456.
[11] C. Almazán, K.M. Kocan, D.K. Bergman, J.C. Garcia-Garcia, E.F. Blouin, J. de la Fuente, Identification of protective antigens for the control of *Ixodes scapularis* infestations using cDNA expression library immunization, Vaccine 21 (2003) 1492–1501.
[12] C. Almazán, K.M. Kocan, D.K. Bergman, J.C. Garcia-Garcia, E.F. Blouin, J. de la Fuente, Characterization of genes transcribed in an *Ixodes scapularis* cell line that were identified by expression library immunization and analysis of expressed sequence tags, Gene Ther. Mol. Biol. 7 (2003) 43–59.
[13] C. Almazán, U. Blas-Machado, K.M. Kocan, J.H. Yoshioka, E.F. Blouin, A.J. Mangold, J. de la Fuente, Characterization of three *Ixodes scapularis* cDNAs protective against tick infestations, Vaccine 23 (2005) 4403–4416.
[14] C. Almazán, K.M. Kocan, E.F. Blouin, J. de la Fuente, Vaccination with recombinant tick antigens for the control of *Ixodes scapularis* adult infestations, Vaccine 23 (2005) 5294–5298.
[15] J. de la Fuente, C. Almazán, E.F. Blouin, V. Naranjo, K.M. Kocan, RNA interference screening in ticks for identification of protective antigens, Parasitol. Res. 96 (2005) 137–141.
[16] J. de la Fuente, C. Almazán, U. Blas-Machado, V. Naranjo, A.T. Mangold, E.F. Blouin, K.M. Kocan, The tick protective antigen, 4D8, is a conserved protein involved in modulation of tick blood digestion and reproduction, Vaccin (2006), doi:10.1016/j.vaccine.2006.02.046.
[17] R.L. Osburn, E.F. Knipling, The potential use of sterile hybrid *Boophilus* ticks (Acari: Ixodidae) as a supplemental eradication technique, J. Med. Entomol. 19 (1982) 637–644.
[18] L.R. Hilburn, R.B. Davey, J.E. George, J.M. Pound, Non-random mating between *Boophilus microplus* and hybrids of *B. microplus* females and *B. annulatus* males, and its possible effect on sterile male hybrid control releases, Exp. Appl. Acarol. 11 (1991) 23–36.
[19] M.N. Aljamali, A.D. Bior, J.R. Sauer, R.C. Essenberg, RNA interference in ticks: a study using histamine binding protein dsRNA in the female tick *Amblyomma americanum*, Insect Mol. Biol. 12 (2003) 299–305.
[20] A.E. Kiszewski, A. Spielman, Preprandial inhibition of re-mating in *Ixodes* ticks (Acari: Ixodidae), J. Med. Entomol. 39 (2002) 847–853.
[21] Y.S. Balashov, Bloodsucking ticks (Ixodoidea)—Vectors of diseases of man and animals, Misc. Pub. Entom. Soc. Am. 8 (1972) 160–376.
[22] R.E. Purnell, J.D. Dargie, B. Gilliver, A.D. Irvin, M.A. Ledger, Some effects of irradiation on the tick *Rhipicephalus appendiculatus*, Parasitology 64 (1972) 429–440.
[23] C.A. Soares, C.M. Lima, M.C. Dolan, J. Piesman, C.B. Beard, N.S. Zeidner, Capillary feeding of specific dsRNA induces silencing of the isac gene in nymphal *Ixodes scapularis* ticks, Insect Mol. Biol. 14 (2005) 443–452.
[24] K.M. Kocan, J.H. Yoshioka, D.E. Sonenshine, J. de la Fuente, S.M. Ceraul, E.F. Blouin, C. Almazán, Capillary tube feeding system for studying tick-pathogen interactions of *Dermacentor variabilis* (Acari: Ixodidae) and *Anaplasma marginale* (Rickettsiales: Anaplasmataceae), J. Med. Entomol. 42 (2005) 864–874.