ELECTRONIC APPENDIX

This is the Electronic Appendix to the article

Sperm competition and the evolution of male reproductive anatomy in rodents

by

Steven A. Ramm, Geoffrey A. Parker and Paula Stockley

Proc. R. Soc. B (doi:10.1098/rspb.2004.3048)

Sperm competition and the evolution of male reproductive anatomy in rodents

Steven A. Ramm, Geoffrey A. Parker and Paula Stockley

Supplementary information

Appendix A

Phylogenies used for comparative analyses.

Appendix B

Relative testis sizes and prevalence of within-litter multiple paternity in rodents.

Appendix C

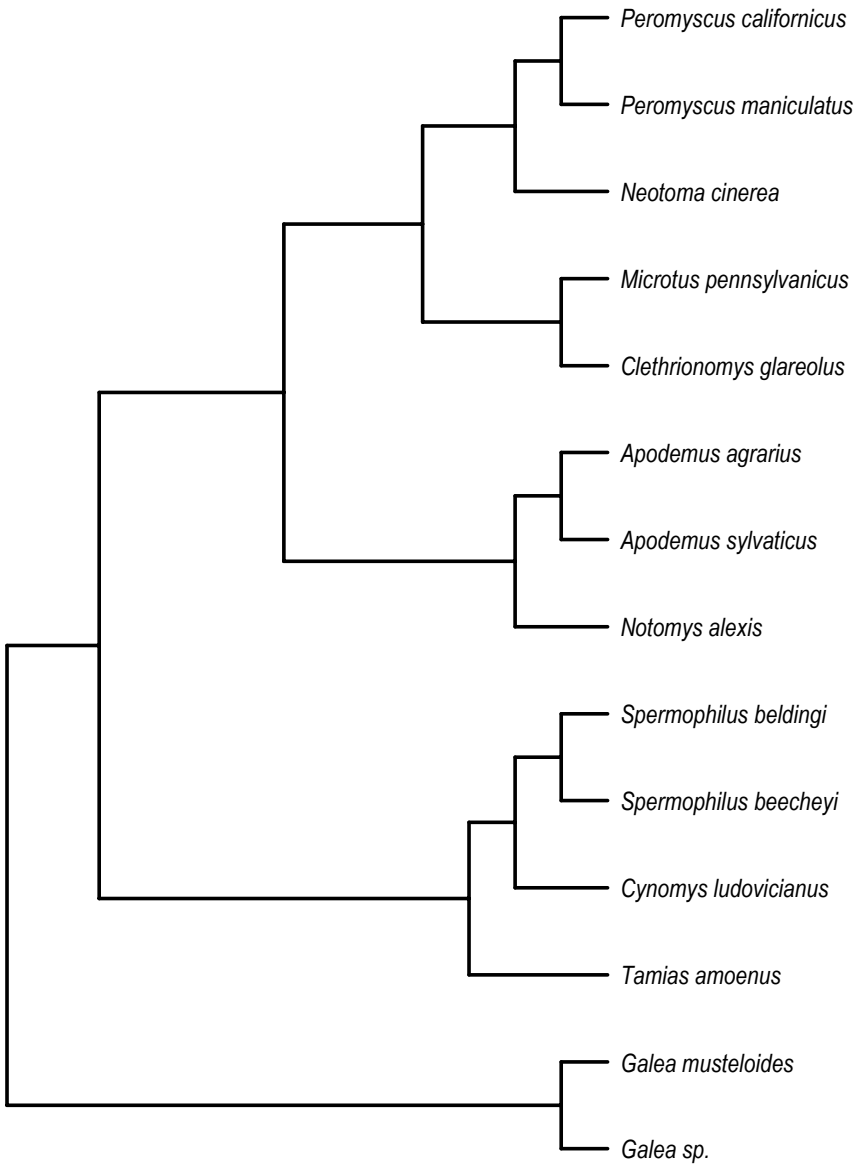
Body, testis and accessory reproductive gland masses of 42 rodent species.

Appendix D

Copulatory plug size, vaginal length and relative testis size in rodents.

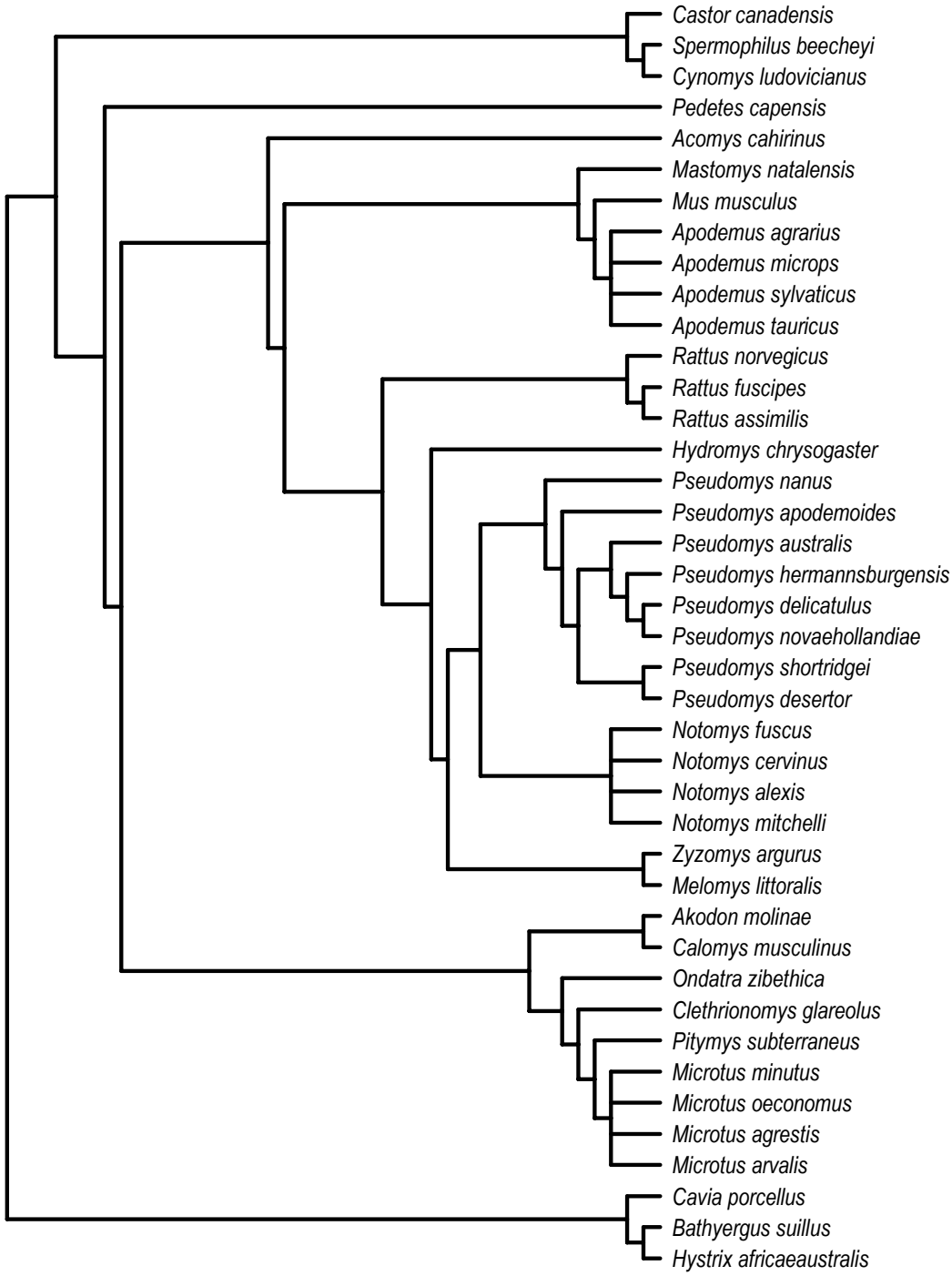
Appendix A (i)

Phylogeny used for GLS analysis of relative testis size and multiple paternity data.



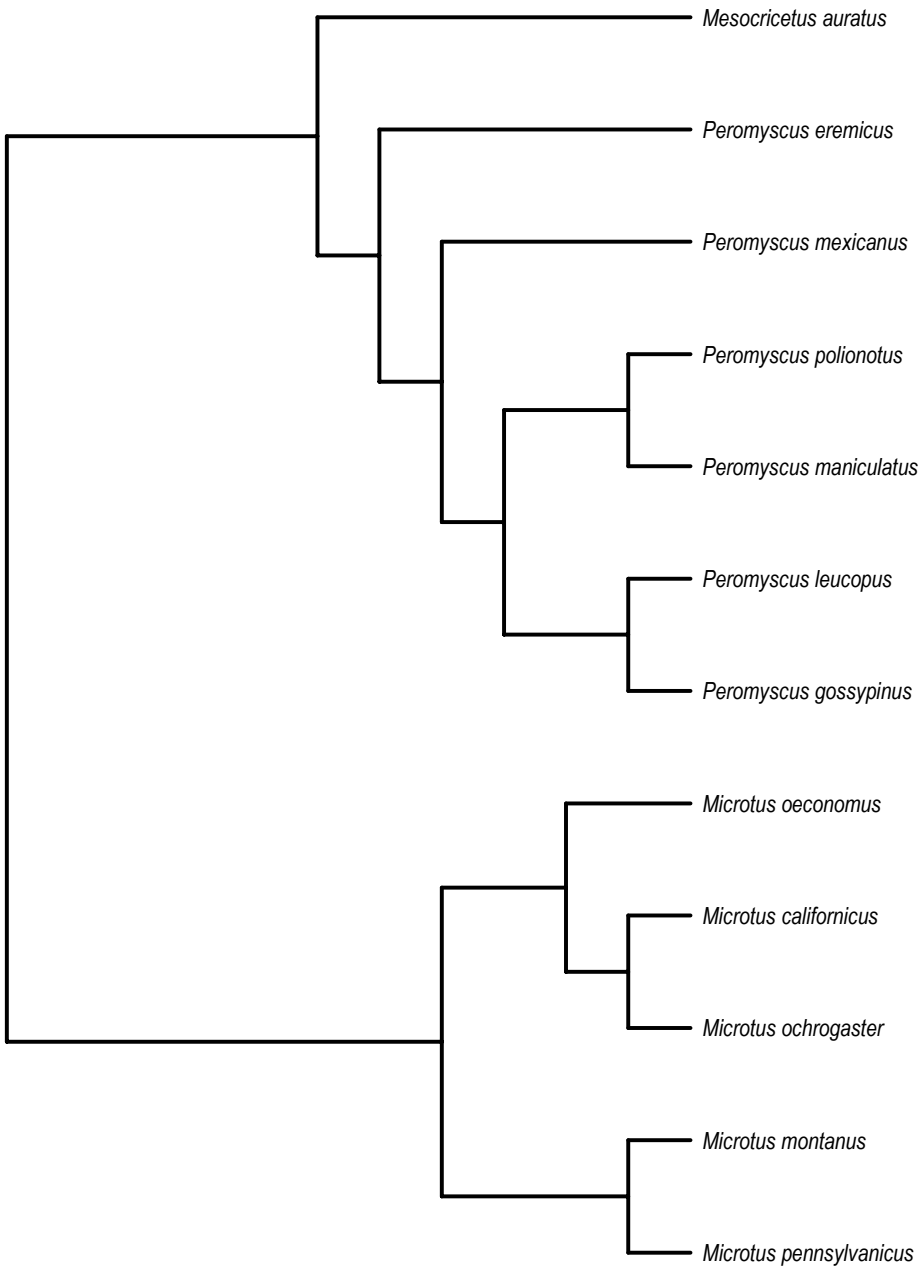
Appendix A (ii)

Phylogeny used for GLS analysis of accessory glands data.



Appendix A (iii)

Phylogeny used for GLS analysis of copulatory plug size data.



Phylogenetic sources: Hooper & Musser (1964); Baverstock et al. (1981); Stangl & Baker (1984); Corbet & Hill (1991); Watts et al. (1992); Martin et al. (2000); Liu et al. (2001); Herron et al. (2004); Steppan et al. (2004).

Appendix B

Relative testis sizes, prevalence of within-litter multiple paternity and litter size in rodents.

Species	RTS[1]	MP[2]	LS[3]	Source(s)
Apodemus agrarius	2.99	80	6.50	Kenagy & Trombulak 1986; Baker et al. 1999
A. sylvaticus	2.27	50	4.67	Kenagy & Trombulak 1986; Baker et al. 1999
Clethrionomys glareolus	1.94	35.5	4.17	Kenagy & Trombulak 1986; Ratkiewicz & Borkowska 2000; Hayssen et al. 1993
Cynomys ludovicianus	0.24	3.95	3.88	Foreman 1998; Hoogland & Foltz 1982, Hoogland 1995; Hayssen et al. 1993
Galea musteloides	2.33	83.3	3.09	Sachser et al. 1999; Hayssen et al. 1993
G. sp.	1.62	0	1.28[4]	Hohoff et al. 2002
Microtus pennsylvanicus	1.73	14.6	4.88	Pierce et al. 1990; Boonstra et al. 1993; Hayssen et al. 1993
Neotoma cinerea	0.66	0	3.40	A. Schulte-Hostedde, unpubl. data; Topping & Millar 1998; Hayssen et al. 1993
Notomys alexis	0.11	0	4.00	Kenagy & Trombulak 1986; Breed & Adams 1992; Hayssen et al. 1993
Peromyscus californicus	0.02	0	2.03	Nelson et al. 1995; Ribble 1991; Hayssen et al. 1993
P. maniculatus	1.29	10.3	4.78	Kenagy & Trombulak 1986; Birdsall & Nash 1973
Spermophilus beecheyi	1.64	89.0	5.62	Kenagy & Trombulak 1986; Boellstorf et al. 1994; Hayssen et al. 1993
S. beldingi	1.20	77.8	5.70	McKeever 1963; Hanken & Sherman 1981; Hayssen et al. 1993
Tamias amoenus	1.11	59.5	5.32	Schulte-Hostedde & Millar 2004; Schulte-Hostedde et al. 2004; Hayssen et al. 1993

[1] relative testis size (based on the rodent regression equation calculated by Kenagy and Trombulak 1986);
[2] percenatge of litters reported with multiple paternity.
[3] litter size.
[4] minimum litter size from data reported.

Appendix C

Body, testis and accessory reproductive gland masses of 43 rodent species.

Species	*n*	BM[1] (g)	TM[2] (g)	SVM[3] (g)	APM[4] (g)	VPM[5] (g)	Source(s)
Acomys cahirinus	5	75.5	0.512	0.2711	0.0295	0.0378	Dewsbury & Hodges 1987; Peitz et al. 1979
Akodon molinae	10	41.49	0.446	0.23	0.0289	0.0542	Yunes & Castro-Vazquez 1990
Apodemus agrarius	141	21.8	0.994			0.0234	Humiński 1969
A. microps	8	19.2	0.326			0.0108	Humiński 1969
A. sylvaticus[6]	33	23.1	0.788			0.026	Humiński 1969
A. sylvaticus[6]	5	26.4	1.397	0.578			Eriksson 1981
A. tauricus	61	29.1	0.891			0.0243	Humiński 1969
Bathyergus suillus	4	750	2.205	0.6488			Van der Horst 1972
Calomys musculinus	8	35.37	0.1979	0.1345	0.0407	0.0439	Buzzio and Castro-Vazquez 2002
Castor canadensis	31	36480	9.3	13.68			Osborn 1953
Cavia porcellus	18	714.5	3.73	3.1			Warnock 1923
Clethrionomys glareolus	87	21.8	0.646			0.0442	Humiński 1969
Cynomys ludovicianus	35	815	4	0.9			Kenagy & Trombulak 1986; Anthony 1953
Hydromys chrysogaster	13	897.32	8.471	2.993			Olsen 1982
Hystrix africaeaustralis	124	11790	6.649	5.067			Van Aarde & Skinner 1986
Mastomys natalensis		49.2	1.19	0.36			Silva & Downing 1995; Neal 1977
Melomys littoralis		61	1.684			0.063	Breed & Sarafis 1978
Micromys minutus	9	7.8	0.178			0.0065	Humiński 1969
Microtus agrestis	31	34.35	0.5406	0.238		0.0395	Clarke & Forsyth 1964
M. arvalis[6]	395	28.5	0.424			0.041	Humiński 1958
M. arvalis[6]		21.2	0.199	0.175		0.008	Delost 1951
M. oeconomus	10	34.8	0.419			0.0372	Humiński 1969
Mus musculus[6]	6	21.1	0.181	0.303			S. A. Ramm, unpubl. data
M. musculus[6]	36	15.3	0.119			0.008	Humiński 1969
Notomys alexis	12	34	0.05			0.105	Breed 1986
N. cervinus	2	33	0.198	0.182	0.025	0.106	Breed 1986
N. fuscus	1	44	0.062			0.09	Breed 1986

Appendix C *contd.*

Species	*n*	BM[1] (g)	TM[2] (g)	SVM[3] (g)	APM[4] (g)	VPM[5] (g)	Source(s)
N. mitchelli	8	39	0.062			0.174	Breed 1982
Ondatra zibethica	5	1105	5.064	4.887	0.259		Beer & Meyer 1951
Pedetes capensis	93	3230	18.24	9.6			Butynski 1979
Pitymys subterraneus	17	17.1	0.237			0.055	Humiński 1969
Pseudomys apodemoides	7	31	0.141	0.142	0.018	0.012	Breed 1982
P. australis	13	59	2.242	0.865	0.272	0.09	Breed 1982
P. delicatulus	3	8	0.05	0.032	0.018	0.01	Breed 1982
P. desertor	1	39	0.558	0.202	0.056	0.022	Breed 1982
P. hermannsburgensis	7	18	0.163	0.122	0.022	0.048	Breed 1982
P. nanus	6	78	1.874	0.694	0.257	0.097	Breed 1982
P. novaehollandiae	2	19	0.071	0.086	0.032	0.065	Breed 1982
P. shortridgei	2	78	0.345	0.222	0.06	0.02	Breed 1982
Rattus assimilis		115	4.8	1			Taylor 1961
R. fuscipes		100	4.41			0.247	Breed & Sarafis 1978
R. norvegicus	19	500	4	1.115			Pessah & Kochva 1975
Spermophilus beecheyi	67	626	6.657	1.897			Tomich 1962
S. beldingi	29	210	0.795	0.94			McKeever 1963
Zyzomys argurus		53	0.4			0.043	Breed & Sarafis 1978

[1] body mass;
[2] paired testis mass;
[3] paired seminal vesicles mass;
[4] paired anterior prostate mass;
[5] paired ventral prostate mass;
[6] differing testis and body mass data reported in studies measuring different accessory glands.

Appendix D

Copulatory plug size, vaginal length and relative testis size in rodents.

Species	PL[1]	VL[2]	RTS[3]	RTS Source(s)
Microtus californicus	4.9	7.4	0.35	Kenagy & Trombulak 1986
M. montanus	5.9	8.7	0.68	Pierce et al. 1990
M. ochrogaster	5.8	8.5	0.88	Pierce et al. 1990
M. oeconomus	6.1	9.3	0.88	Kenagy & Trombulak 1986
M. pennsylvanicus	9.2	10.1	1.73	Pierce et al. 1990
Peromyscus eremicus	7.5	12.1	0.64	Linzey & Layne 1969; Silva & Downing 1995
P. gossypinus	6.4	10.1	1.54	Linzey & Layne 1969; Silva & Downing 1995
P. leucopus	8.0	10.3	0.77	Linzey & Layne 1969; Silva & Downing 1995
P. maniculatus	8.0	9.1	1.29	Kenagy & Trombulak 1986
P. mexicanus	10.5	15.5	0.94	Linzey & Layne 1969; Silva & Downing 1995
P. polionotus	7.5	10.0	0.43	Linzey & Layne 1969; Silva & Downing 1995
Mesocricetus auratus	9.0	11.3	2.78	Kenagy & Trombulak 1986

[1] copulatory plug length (mm), from Baumgardner et al. (1982);
[2] vaginal length (mm), from Baumgardner et al. (1982);
[3] relative testis size (based on the rodent regression equation calculated by Kenagy and Trombulak 1986).

References

Anthony, A. 1953 Seasonal reproductive cycle in the normal and experimentally treated male prairie dog, *Cynomys ludovicianus*. *J. Morphol.* **93**, 331-369.

Baker, R. J., Makova, K. D. & Chesser, R. K. 1999 Microsatellites indicate a high frequency of multiple paternity in *Apodemus* (Rodentia). *Mol. Ecol.* **8**, 107-111.

Baumgardner, D. J., Hartung, T. G., Sawrey, D. K., Webster, D. G. & Dewsbury, D. A. 1982 Muroid copulatory plugs and female reproductive tracts: a comparative investigation. *J. Mammal.* **63**, 110-117.

Baverstock, P. R., Watts, C. H. S., Adams, M. & Cole, S. R. 1981 Genetical relationships among Australian rodents (Muridae). *Aust. J. Zool*. **29**, 289-303.

Birdsall, D. A. & Nash, D. 1973 Occurrence of successful multiple insemination of females in natural populations of deer mice (*Peromyscus maniculatus*). *Evolution* **27**, 106-110.

Boellstorff, D. E , Owings, D. H., Penedo, M. C. T. & Hersek, M. J. 1994 Reproductive behaviour and multiple paternity of California ground squirrels. *Anim. Behav.* **47**, 1057-1064.

Boonstra, R., Xia, X. & Pavone, L. 1993 Mating system of the meadow vole, *Microtus pennsylvanicus*. *Behav. Ecol.* **4**, 83-89.

Beer, J. R. & Meyer, R. K. 1951 Seasonal changes in the endocrine organs and behavior patterns of the muskrat. *J. Mammal.* **32**, 173-191.

Breed, W. G. 1982 Morphological variation in the testes and accessory sex organs of Australian rodents in the genera *Pseudomys* and *Notomys*. *J. Reprod. Fertil.* **66**, 607-613.

Breed, W. G. 1986 Comparative morphology and evolution of the male reproductive tract in the Australian hydromyine rodents (*Muridae*). *J. Zool. Lond.* A **209**, 607-629.

Breed, W. G. & Adams, M., 1992 Breeding systems of Spinifex hopping mice (*Notomys alexis*) and plains rats (*Pseudomys australis*): a test of multiple paternity within the laboratory. *Aust. J. Zool.* **40**, 13-20.

Breed, W. G. & Sarafis, V. 1978 On the phylogenetic significance of spermatozoal morphology and male reproductive tract anatomy in Australian rodents. *Trans. R. Soc. S. Aust.* **103**, 127-135.

Butynski, T. M. 1979 Reproductive ecology of the springhaas *Pedetes capensis* in Botswana. *J. Zool.* **189**, 221-232.

Buzzio, O. L. & Castro-Vazquez, A. 2002 Reproductive biology of the corn mouse, *Calomys musculinus*, a neotropical sigmodontine. *J. Neotrop. Mammal.* **9**, 135-158.

Clarke, J. R. & Forsyth, I. A. 1964 Seasonal changes in the gonads and accessory reproductive organs of the vole (*Microtus agrestis*). *Gen. Comp. Endocrinol.* **4**, 233-242.

Corbet, G. B. & Hill, J. E. 1991 *A world list of mammalian species*, 3rd ed. Oxford University Press.

Delost, P. 1951 État de l'appareil génital du Campagnol des champs (*Microtus arvalis*) de sexe mâle en hiver. *Bull. Soc. Hist. Nat. Toulouse* **86**, 133-150.

Dewsbury, D. A. & Hodges, A. W. 1987 Copulatory behaviour and related phenomena in spiny mice (*Acomys cahirinus*) and hopping mice (*Notomys alexis*). *J. Mammal.* **68**, 49-57.

Eriksson, M. 1981 The bulbourethral gland of the wood mouse, *Apodemus sylvaticus*. *J. Mammal.* **62**, 375-378.

Foreman, D. 1998 Effects of exogenous hormones on spermatogenesis in the male prairie dog (*Cynomys ludovicianus*). *Anat. Rec.* **250**, 45-61.

Hanken, J. & Sherman, P. W., 1981 Multiple paternity in Belding's ground squirrels. *Science* **212**, 351-353.

Hayssen, V., van Tienhoven, A. & van Tienhoven, A. 1993 *Asdell's patterns of mammalian reproduction*. London: Comstock.

Herron, M. D., Castoe, T. A. & Parkinson, C. L. 2004 Sciurid phylogeny and the paraphyly of Holarctic ground squirrels (Spermophilus). *Mol. Phylogenet. Evol.* **31**, 1015-1030.

Hohoff, C., Solmsdorff, K., Löttker, P., Kemme, K., Epplen, J. T., Cooper, T. G. & Sachser, N. 2002 Monogamy in a new species of wild guinea pigs (*Galea* sp.). *Naturwissenschaften* **89**, 462-465.

Hoogland, J. L. 1995 *The black-tailed prairie dog: social life of a burrowing mammal*. University of Chicago Press.

Hoogland, J. L. & Foltz, D. W. 1982 Variance in male and female reproductive success in a harem-polygynous mammal, the black-tailed prairie dog (Sciuridae: *Cynomys ludovicianus*). *Behav. Ecol. Sociobiol.* **11**, 155-163.

Hooper, E. T. & Musser, G. G. 1964 The glans penis in neotropical cricetines (family Muridae) with comments on classification of muroid rodents. *Misc. Publ. Mus. Zool. Univ. Mich.* **123**, 1-57.

Humiński, S. 1958 The autumnal involution of the male sexual apparatus in the field vole (*Microtus arvalis* Pall.). *Zool. Pol.* **9**, 197-214.

Humiński, S. 1969 Biomorphological studies on testes and male accessory glands in some species of the families *Muridae* and *Microtidae* found in Poland. *Zool. Pol.* **19**, 213-255.

Kenagy, G. J. & Trombulak, S. C. 1986 Size and function of mammalian testes in relation to body size. *J. Mammal.* **67**, 1-22.

Linzey, A. V. & Layne, J. N. 1969 Comparative morphology of the male reproductive tract in the rodent genus *Peromyscus* (Muridae). *Amer. Mus. Nov.* **2355**, 1-47.

Liu, F.-G. R., Miyamoto, M.M., Freire, N. P., Ong, P. Q., Tennant, M. R., Young, T. S. & Gugel, K. F. 2001 Molecular and morphological supertrees for Eutherian (placental) mammals. *Science* **291**, 1786-1789.

Martin, Y., Gerlach, G., Schlotterer, C. & Meyer, A. 2000 Molecular phylogeny of European muroid rodents based on complete cytochrome b sequences. *Mol. Phylogenet. Evol.* **16**, 37-47.

McKeever, S. 1963 Seasonal changes in body weight, reproductive organs, pituitary, adrenal glands, thyroid gland, and spleen of the Belding ground squirrel (*Citellus beldingi*). *Am. J. Anat.* **113**, 153-173.

Neal, B. R. 1977 Reproduction of the multimammate rat, *Praomys* (*Mastomys*) *natalensis* (Smith), in Uganda. *Z. Säugetierkunde* **42**, 221-231.

Nelson, R. J., Gubernick, D. J. & Blom, J. M. C. 1995 Influence of photoperiod, green food, and water availability on reproduction in male Californian mice (*Peromyscus californicus*). *Physiol. Behav.* **57**, 1175-1180.

Olsen, P. D. 1982 Reproductive biology and development of the water rat, *Hydromys chrysogaster*, in captivity. *Aust. Wildl. Res.* **9**, 39-53.

Osborn, D. J. 1953 Age classes, reproduction, and sex ratios of Wyoming beaver. *J. Mammal.* **34**, 27-44.

Pierce, J. D., Jr, Ferguson, B., Salo, A. L., Sawrey, D. K., Shapiro, L. E., Taylor, S. A. & Dewsbury, D. A. 1990 Patterns of sperm allocation across successive ejaculates in four species of voles (*Microtus*). *J. Reprod. Fertil.* **88**, 141-149.

Peitz, B., Foreman, D. & Schmitt, M. 1979 The reproductive tract of the male spiny mouse (*Acomys cahirinus*) and coagulation studies with other species. *J. Reprod. Fertil.* **57**, 183-188.

Pessah, H. & Kochva, E. 1975 The secretory activity of the seminal vesicles in the rat after copulation. *Biol. Reprod.* **13**, 557-560.

Pierce, J. D., Jr, Ferguson, B., Salo, A. L., Sawrey, D. K., Shapiro, L. E., Taylor, S. A. & Dewsbury, D. A. 1990 Patterns of sperm allocation across successive ejaculates in four species of voles (*Microtus*). *J. Reprod. Fertil.* **88**, 141-149.

Ratkiewicz, M. & Borkowska, A. 2000 Multiple paternity in the bank vole (*Clethrionomys glareolus*): field and experimental data. *Z. Saugetierkd.* **65**, 6-14.

Ribble, D. O. 1991 The monogamous mating system of *Peromyscus californicus* as revealed by DNA fingerprinting. *Behav. Ecol. Sociobiol.* **29**, 161-166.

Sachser, N., Schwarz-Weig, E., Keil, A. & Epplen, J. T. 1999 Behavioural strategies, testis size, and reproductive success in two caviomorph rodents with different mating systems. *Behav*. **136** 1203-1217.

Schulte-Hostedde, A. I. & Millar, J. S. 2004 Intraspecific variation of testis size and sperm length in the yellow-pine chipmunk (*Tamias amoenus*): implications for sperm competition and reproductive success. *Behav. Ecol. Sociobiol.* **55**, 272-277.

Schulte-Hostedde, A. I., Millar, J. S. & Gibbs, H. L., 2004 Sexual selection and mating patterns in a mammal with female-biased sexual size dimorphism. *Behav. Ecol.* **15**, 351-356.

Silva, M. & Downing, J. A. 1995 *CRC Handbook of Mammalian Body Masses*. Boca Raton, Fla: CRC Press.

Stangl, F. B. & Baker, R. J. 1984 Evolutionary relationships in *Peromyscus*: congruence in chromosomal, genic, and classical data sets. *J. Mamm.* **65**, 643-654.

Steppan, S. J., Adkins, R. M. & Anderson, J. 2004 Phylogeny and divergence-date estimates of rapid radiations in muroid rodents based on multiple nuclear genes. *Syst. Biol.* (in press).

Taylor, J. M. 1961 Reproductive biology of the Australian bush rat, *Rattus assimilis*. *Univ. Calif. Publ. Zool.* **60**, 1-66.

Tomich, P. Q. 1962 The annual cycle of the California ground squirrel *Citellus beecheyi*. *Univ. Calif. Publ. Zool.* **65**, 213-282.

Topping, M. G. & Millar, J. S. 1998 Mating patterns and reproductive success in the bushy-tailed woodrat (*Neotoma cinerea*), as revealed by DNA fingerprinting. *Behav. Ecol. Sociobiol.* **43**, 115-124.

Van Aarde, R. J. & Skinner, J. D. 1986 Reproductive biology of the male Cape porcupine, *Hystrix africaeaustralis*. *J. Reprod. Fertil.* **76**, 545-552.

Van der Horst, G. 1972 Seasonal effects on the anatomy and histology of the reproductive tract of the male rodent mole *Bathyergus suillus suillus* (Schreber). *Zool. Afr.* **7**, 491-520.

Warnock, A. W. 1923 The anatomy of the seminal vesicles of the guinea-pig, *Cavia cobaya. Anat. Rec.* **25**, 275-287.

Watts, C. H. S. & Baverstock, P. R., Birrell, J. & Krieg, M. 1992 Phylogeny of the Australian rodents (Muridae): a molecular approach using microcomplement fixation of albumin. *Aust. J. Zool.* **40**, 81-90.

Yunes, R. M. F. & Castro-Vazquez, A. 1990 An unusual pattern of copulatory behavior in a South American cricetid rodent: *Akodon molinae*. *J. Comp. Psychol.* **104**, 263-267.