

The Influence of Parasite Infection on Mating Success in Spadefoot Toads, *Scaphiopus couchii*¹

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SYNOPSIS. The desert toads, *Scaphiopus couchii*, have an annual activity season of less than 8 weeks and experience only one significant parasite infection: the monogenean *Pseudodiploorchis americanus* is transmitted during host spawning and provides a natural system for testing the influence of parasite burden on host mating success. The 10 month hibernation involves total starvation during which the blood-feeding parasites reduce fat reserves and haematocrit. The toads emerge and spawn on the first night after rainfall, before they replenish depleted reserves. Male chorusing is energetically very demanding and mate selection, limited to a 7 hr nocturnal assembly, is determined principally by female choice. Parasite transmission triggered by host sexual activity results in 100% prevalence and high intensities of infection amongst males. Around 50% of toads lose their burdens entirely but the rest carry chronic infections throughout host hibernation. Field data show a consistent reduction in intensity each year strongly suggesting resistance. Parasite infection is pathogenic and creates extra stress during hibernation; therefore, to the extent that elimination of infection is heritable, toads entering spawning assemblies with heavy burdens should make poor mates. However, extensive field studies show no correlation between mate success and parasite burden. Although infection can prejudice survival, it is only one of several inter-related factors (including feeding success, tolerance of hibernation). The condition of successful and unsuccessful males in spawning assemblies indicates that all exceed a threshold at which parasite-induced pathology is significant. Males which are debilitated by infection—or other factors—are selected against before mate choice begins.

INTRODUCTION

The secondary sexual characters of males are assumed to have evolved through female preference, yet these traits are often apparently deleterious, involving high cost in resource investment and increased predation risk. Hamilton and Zuk (1982) proposed that females use secondary sexual characters as indicators of a male's genetic ability to resist disease since the full expression of these displays depends on health and vigour. By mating with such males, females increase the fitness of their offspring through improved disease resistance. Two predictions follow from this model. In species particularly vulnerable to infection, characters will have been selected which allow females to assess parasite burden and disease resistance; so, comparative surveys between species should reveal that the development of secondary sexual characters correlates posi-

tively with infection. Secondly, within a species, there should be an inverse correlation between male mating success and individual parasite load.

The Hamilton and Zuk hypothesis has stimulated much debate and research at the interface of parasitology and evolutionary biology. Most empirical investigations have approached the subject from a standpoint within animal behaviour, based on the observable traits which provide a mechanism for mate selection. However, there has been a tendency for parasites to be incorporated into some of these analyses as statistical abstractions, without reference to the highly variable nature of host-parasite relationships. Relatively few studies have incorporated detailed information on the interactions of the parasite with the host, including such basic aspects as the distribution of infection levels within host populations and the pathological consequences of infection. Virtually without exception, the systems examined provide no information on the genetic basis of resistance; this reflects the fact that parasitological research has only recently

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begun to document the variation in immunological characteristics within natural animal populations. Much of the current debate concerns the appropriateness of different host-parasite systems for testing the hypothesis. This field and laboratory study is based on a desert amphibian which permits a very rigorous test supported by comprehensive information on host and parasite biology.

Characteristics of the system: The host

The North American spadefoot toads, *Scaphiopus* species, are superbly adapted to arid environments and their ecophysiological specialisations have been documented in detail (see McClanahan, 1967; Ruibal *et al.*, 1969; Dimmitt and Ruibal, 1980a, b; and references). The toads avoid the harshest desert conditions by hibernating up to a metre below the soil surface. They emerge on the first night of heavy rains, generally in early July in Arizona, and spawn in the newly-formed pools. They then feed on a variety of desert invertebrates during favourable conditions lasting little more than 8 weeks. However, their real activity season is invariably much less: The nocturnal toads only actually emerge to forage on nights following rainfall when the desert surface is relatively damp. Otherwise, they remain buried a few centimetres below ground. As a result, surface activity probably occurs on fewer than 20 nights each year. In September, the toads retreat into deep hibernation burrows where they are inactive for the next 9 or 10 months, surviving on fat reserves accumulated during their brief feeding.

The "monsoons" in S.E. Arizona typically begin suddenly with torrential rainfall, and within hours accumulation of run-off water provides spawning sites for the spadefoot toads. Analysis of gut contents of toads entering "first night" spawning assemblies shows that they rarely feed *en route* to water (Tinsley and Jackson, 1988). Mating assemblies of *S. couchii* begin to form at darkness, around 21.00 hr, but spawning ends abruptly at dawn, around 04.00 hr, when the toads leave the pools and bury themselves to avoid the daytime heat. Female *S. couchii* enter breeding sites on

only one night in the season and they complete spawning and leave within 3 or 4 hr. Males remain in water from their time of arrival until the assembly disperses, a maximum of 7 hr. If there are subsequent rains heavy enough to produce spawning pools, some males may re-enter and will pair with different subsets of the female population which did not breed on the previous occasion. However, there are rarely more than three opportunities for spawning, giving males less than 24 hr each year to obtain a mate (Tinsley, 1989, 1990).

Males outnumber females by between 2:1 and 10:1, and competition is very intense. Chorusing is an extremely exhausting activity and, after an assembly, males may have visible haemorrhages on the delicate vocal sac. The very high metabolic cost of anuran vocalisation has been quantified by Taigen and Wells (1985) for *Hyla versicolor*, and spadefoot toad calling seems to exhibit similar characteristics. The term "scramble competition" which has been applied to some explosively-breeding anurans is misleading in the case of *S. couchii*. The majority of males call whilst floating and some whilst sitting in shallow water, occasionally moving to new positions to resume calling. Some males show satellite behaviour, floating silently near a calling male, and some engage in active searching, wrestling with any toad encountered. Nevertheless, as a rule, it is the female who selects the male. Faced with often dense aggregations of males, a female will swim past a succession of calling individuals apparently in a direct movement towards a chosen male. This male remains unresponsive until he is nudged by the female and this acts as a signal for him to engage in amplexus. Competing males may attempt to dislodge an amplexing male but these are always kicked away before they can secure a hold: I have never seen an amplexing male displaced; Sullivan (1985) concurs.

Characteristics of the system: Infection

The elusive life style of the spadefoot toads imposes severe constraints on parasite transmission. For helminths with indirect life cycles, employing at least one

intermediate host, there are limited opportunities during the brief activity season for infective stages to be released from one toad, to be ingested by and develop within an invertebrate host, and for this to be eaten by another toad. Some helminths which are common parasites of other anurans are absent: the lack of digeneans is related to the absence of snails which are obligatory intermediate hosts in their life cycles. These limitations result in a very restricted parasite fauna. At my Arizona study sites, *Scaphiopus couchii* carries opalinid and ciliate protozoans (in the rectum) and 5 species of helminths (Tinsley, unpublished observations). Two nematodes occur in the gut: *Aplectana itzocamensis* in the rectum and *Physaloptera* sp. in the stomach; microfilariae (larvae of an unidentified filarial nematode) are carried in the bloodstream; and a tapeworm, *Distoichometra bufonis*, infects the duodenum. All of these occur infrequently, with a prevalence of infection less than 5%, and generally very low intensities: the cestode, for instance, is rarely represented by more than one worm per host. Only one parasite is common: a monogenean, *Pseudodiplorchis americanus*, infects more than 50% of *S. couchii* with a mean of around 5 adult worms per infected toad, and burdens of juvenile parasites may exceed 300 worms per host (Tinsley and Jackson, 1988; Tinsley, 1989). The association between *S. couchii* and its single, pervasive, monogenean parasite provides a very precisely organised system for a within-species test of the Hamilton and Zuk model.

P. americanus has a direct life cycle (involving only one host) and transmission is effected by a swimming ciliated infective stage, the oncomiracidium. In consequence, infection is restricted exclusively to the period when the toads enter water to spawn. The adult worms, which live in the urinary bladder, respond to this fleeting opportunity by a strategy involving ovoviviparity (Tinsley, 1983a). About 100 offspring are retained within the parasite's uterus and develop to an infective stage during the long period of host hibernation. When the toads enter spawning sites, oncomiracidia are discharged *en masse* with

the host's urine, and immediately invade other toads congregating in the same pool. The life cycle is unique amongst platyhelminths. Larvae invade the toads via the nostrils and develop within the respiratory tract for about a month. Then they migrate via the stomach and intestine to the bladder where they reach maturity and begin to manufacture offspring *in utero* for transmission in the next summer (Tinsley and Earle, 1983; Tinsley and Jackson, 1986).

The entire life cycle of *P. americanus* is intimately integrated with host biology, and a vital requirement is that the parasite must concentrate release of its total annual reproductive output into a period of only 7 hr. Field and laboratory experiments (Tinsley, in preparation) have established that the discharge of infective stages is triggered very precisely during host spawning, but the timing of the response differs in the two host sexes. In lab experiments, release of larvae by parasites within a male begins as the host initiates amplexus and continues steadily through the period of pairing. Infected females do not discharge oncomiracidia during this initial amplexus: their parasites respond with a pulse of larval output just as the female starts to release spawn and they stop as spawn output stops. However, a majority of males in an assembly fail to reproduce, what about their parasites? When infected males were put together in groups in aquaria and became sexually aroused, attempting to amplex one another, their parasites released most of their larvae. Intense sexual excitement provides a trigger for the parasite which is as effective as the spawning process. However, in one laboratory trial involving a group of 4 males, there was a negligible response from their parasites even though, at dissection, they contained over 2,000 oncomiracidia which had remained undischarged while the hosts were in water. It was found that these males were in very poor condition after laboratory hibernation, with exhausted fat reserves and no development of secondary sex characters. Since the hosts had failed to become sexually aroused, their parasites had not received the cue for transmission.

The difference in the stimulus for par-

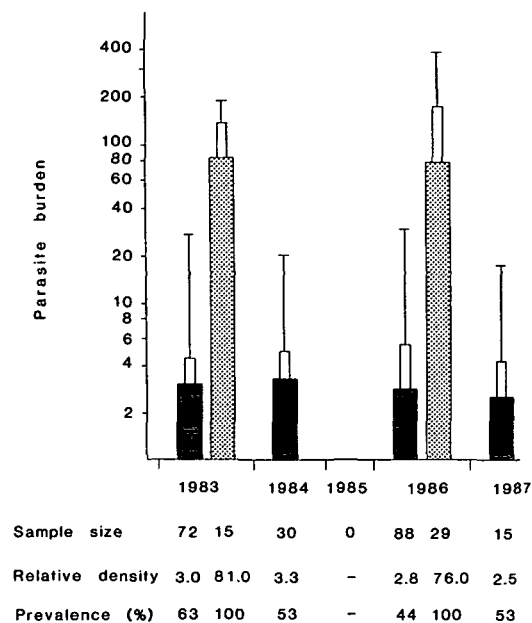


FIG. 1. Infections of *Pseudodiploorchis americanus* in a single population of *Scaphiopus couchii*, showing massive larval invasions (hatched bars) accompanying host spawning, followed by reduction to relatively uniform burdens of adult worms (solid bars).

asite oviposition in male and female hosts maximises transmission potential. Within a mating assembly all females will spawn, so a response triggered during the discharge of ova will be infallible. On the other hand, only a fraction of males will mate, but a cue linked with sexual behaviour guarantees that the parasites in all active males will contribute to transmission.

Host-parasite interactions: Worm burdens

The dynamics of transmission amongst spawning populations of *S. couchii* in S.E. Arizona has been documented by Tinsley and Jackson (1988) for a season in which there were 3 major rainstorms, providing 3 opportunities for breeding (and parasite infection). Amongst males, the 3 exposures resulted in a 100% prevalence and a cumulative mean intensity of over 80 newly-invaded parasites/host. Amongst females, the worm burdens were highly variable (probably reflecting variations in the timing of the brief entry into water) and aver-

aged around 40 parasites/host. The data for male *S. couchii* show that no toad escapes infection (Tinsley and Jackson, 1988). However, while the initial burdens are a product of the ecological circumstances of transmission, the subsequent fate of the parasite population is influenced by host physiology. In the months following invasion, very heavy burdens disappear: the maximum drops from over 300 worms/host (after invasion) to less than 30/host (by the next transmission season, a year later). The mean among male toads drops from over 80 to around 5 per infected toad. A major part of this reduction occurs whilst the host is hibernating. The possibility that these reductions are due to the differential mortality of heavily-infected toads can largely be excluded: toads infected experimentally with over 300 larvae survive a year's laboratory hibernation in good condition and emerge with rarely more than 30 worms/host.

Records for a single population of male *S. couchii* collected within a 100 m radius of the same spawning site in July, 1983–1987 provide longer term data on the control of *P. americanus* infections (Tinsley, 1989). Figure 1 shows two sets of data (1983 and 1984; 1986 and 1987) separated by the 1985 season which was exceptionally dry with no rain at this site during July. Each set of histograms gives, first, the numbers of adult parasites in toads entering the spawning assemblies (in 1983 and 1986) and responsible for releasing that season's infective stages; second, the numbers of newly-invaded parasites accumulating from the exposure; and third, the numbers of parasites surviving to reproduce a year later (in 1984 and 1987) when the toads enter the next spawning assemblies. In both data sets, worm burdens initiating transmission are closely-comparable (relative density around 3) and give rise to similar, highly-successful invasions (100% prevalence and a mean of around 80 worms/toad in 1983 and 1986). One year later, these burdens are dramatically reduced: in both 1984 and 1987, almost 50% of toads were parasite-free and the relative density had been reduced to only 3 or 4% of the initial, post-invasion levels. In terms of the adult *P.*

americanus population carried by this population of *S. couchii*, despite the massive annual invasion, the parasite burdens which survive to reproduce are remarkably constant.

It is now well-established that the immune system of anuran amphibians compares in essential detail with the defensive mechanisms which are better documented in mammals (Horton and Lackie, 1989). The operation of this system against helminth parasites is largely unstudied in anurans. However, the field and laboratory data for *S. couchii* reveal a consistent reduction in worm burdens (despite wide year-to-year variations in environmental conditions in the desert habitats), this is strongly suggestive that the parasite population is controlled by host-mediated factors. The reduction in worm burdens also has important implications for the degree of parasite-induced damage experienced by the host.

Host-parasite interactions: Pathology

P. americanus feeds on blood and virtually the entire demand for nutrients—for growth and manufacture of infective stages—occurs during the 9–10 month period of host starvation. The pathological consequences have been documented by Tocque (1990) with reference to haematocrit, a measure of the erythrocyte concentrations which the parasite depletes, and fat body weight, the reserve upon which the host depends during starvation. Data have been recorded from toads entering mating assemblies in S.E. Arizona after natural hibernation, and from animals maintained under simulated desert conditions in the laboratory in London. The effects of infection differ in the two sexes. In males, parasites reduce both fat reserves and haematocrit, but haematocrit begins to decrease only when all fat has been eliminated. In females, the demands of ovary production normally reduce fat reserves to a minimum during hibernation, and parasite infection affects haematocrit at a much earlier stage of starvation.

Tocque (1990) divided toads sampled from mating assemblies into groups

reflecting their relative physiological condition based on body weight and length. For males in poorer condition, there is little fat and therefore little difference between infected and uninfected toads. Clearly, a range of influences may be responsible for low fat, and the effects of parasite infection cannot be distinguished from poor feeding success in the previous summer, adverse local conditions for hibernation, etc. For males in better condition (which are heavier in relation to their length), fat is significantly reduced in infected compared with uninfected toads (ANOVA, $n = 138$, $P < 0.01$). Comparison of haematocrit in these same groups of mating assembly males revealed no significant difference between infected and uninfected toads (Student's *t*-test, $n = 177$, $P = 0.35$). Haematocrit was reduced by parasite infection only in laboratory trials where males were left in hibernation for nearly two years (a natural occurrence within the range of *S. couchii* in S.E. California (McClanahan, 1967)). In these extreme circumstances, there are no host reserves to buffer the drain caused by the blood-feeding parasite and the reduction in red cell concentrations is correlated with worm burden.

For females emerging from natural hibernation, fat reserves remain only in a small proportion of individuals (those in the best condition), and their haematocrit shows no reduction with infection. In most other females, fatbody weight is minimal at the end of hibernation, presumably because reserves are diverted into ovary manufacture; in these, without a buffer against depletion of blood cells, there is a significant reduction in haematocrit in infected individuals (Student's *t*-test: $n = 83$, $P = 0.023$) (Tocque, 1990).

Clearly, both host and parasite rely on the same finite energy reserves during hibernation. We might predict that the poorer the condition of the host, the progressively greater the pathogenic effect of infection. Since the toads do not feed before mating, the ability of a male to secure a female must depend on energy reserves carried through the previous 10 months starvation. So, does the extra burden

imposed by parasite infection prejudice mating success?

A test of the Hamilton and Zuk hypothesis

The explosive breeding of *S. couchii* provides special advantages for testing the Hamilton and Zuk hypothesis. First, data on sexual selection are completely comprehensive for a given subset of the female population. Females enter spawning pools only once in a season and must complete mate selection and spawning within a matter of hours. Second, the effects of parasite infection are unambiguous in animals newly-emerged from hibernation. All toads entering a "first night" assembly are comparable with regard to the effects of parasite burden on a chronically-starved host. On later occasions any parasite-induced effects could be masked by the host's opportunity to feed, replenishing reserves and compensating for the effects of infection. Additionally, where breeding opportunities are repeated, a male judged to be unsuccessful on one night might actually be recovering from being very successful on a previous occasion. These complications are inherent in some other field tests of the Hamilton and Zuk hypothesis, but they were avoided in this study by concentrating on mating assemblies which occurred on the first night after hibernation.

Data were gathered to correlate mating success with parasite burden in *S. couchii* populations in the San Simon valley, Arizona, in 1985 and 1986. "First night" mating assemblies were sampled on a succession of dates as scattered thunderstorms triggered spawning at different sites in the desert. Males taken in amplexus were regarded as successful since these are not subsequently displaced by other males. Parasite burdens in these were compared with data for unamplexed males taken in the same area of water at the same time. Clearly, the lack of success of these latter males is relevant only up to the time of sampling, but in some sites a series of samples was taken to establish whether mating success might change as the night proceeds. Sampled toads were transferred from water into friable soil and kept sep-

arate in cloth bags to prevent further parasite transmission and enable correlation with field records of individual mating activity. Prior to autopsy, toads were anaesthetised by immersion in MS222 Sandoz, and body length (snout-vent length), body weight, gonad and fatbody weight, and haematocrit were recorded. Parasite infections were recorded in nostril sinuses, oral cavity, vocal sac, glottis, lungs, stomach, intestine, and urinary bladder (for *Pseudodiplorchis americanus*); stomach (*Physaloptera* sp.), rectum (*Aplectana itzocamensis*, and opalinid and balantidial protozoans), anterior intestine (*Distoichometra bufonis*), and kidneys and pulmonary bloodstream (unidentified filarial nematodes). Data are presented here only for the monogenean *P. americanus*, all other parasites had a prevalence less than 5% and, generally, very low intensities. Two categories of *P. americanus* infection were distinguished: newly-invaded stages in the respiratory tract which had been acquired during the brief period of spawning preceding sampling, and adult and pre-adult stages which had been harboured by the host for one year or more. Only the parasites carried by these toads when they entered the breeding sites (and therefore throughout hibernation) could have an effect on performance, and the records which follow are based on these worm burdens.

RESULTS

The first trials, in 1985, were hindered by a very dry season. Rains on 21 July triggered spawning in a series of adjacent pools and one (approx. area 5 × 25 m) was observed through the night. At 01.30 (22 July), all the males at this site were collected. At this time, males which had been successful during the night were still paired, few toads were arriving, and therefore the remaining unamplexed males were unlikely to succeed in mating before the end of the assembly. The prevalence of infection in this population (20%) was unusually low: if infected males are disadvantaged in mating success, then it could be argued that the excess of uninfected males would promote

even stronger selection against those infected. However, worm burdens in successful and unsuccessful males were virtually identical (Table 1). This study provided a complete record of mating success, but the sample was too limited ($n = 35$) for significant conclusions.

The 1986 season provided a series of opportunities for replicate trials. Samples showed irregular differences in infection levels in mated and unmated toads, but none was significant (Mann-Whitney U -test for non-parametric data). When all the records are combined (Fig. 2 and Table 2), there is close agreement in each of the parameters describing the parasite populations: more-or-less identical means, variance to mean ratios, prevalence, etc. Thus, for *P. americanus*, the only important parasite affecting *S. couchii*, there appears to be no significant effect of infection on male mating success.

DISCUSSION

The rationale of the study

Scaphiopus couchii has a species-poor parasite fauna and 4 of the 5 helminths recorded in S.E. Arizona populations occur very infrequently (fewer than 5% of toads infected). There is no information on potential pathogenic effects of these, but significant damage by two of the species is unlikely. The nematode *Aplectana itzocamensis* ingests rectal contents from which nutrient uptake by the host has been completed. *Distoichometra bufonis*, like other cestodes, is dependent upon soluble nutrients in the intestine; so, during host hibernation, it also experiences chronic starvation and loses virtually all proglottids. The other two nematodes could potentially be more damaging, one attached to the stomach mucosa, the other situated in deep tissues (including the kidneys) are releasing microfilariae into the blood. However, these two parasites are also the rarest in the study sites, occurring overall in only 1 or 2% of *S. couchii* (Tinsley, unpublished).

At least 50%, and probably often over 80% of male *S. couchii* in a breeding chorus fail to secure a mate. It is therefore highly improbable that any of these four parasites could exert a significant effect on the out-

TABLE 1. *Pseudodiplorchis americanus* infection in male *Scaphiopus couchii* (mating assembly, San Simon Arizona; 01.30 hr, 22 July 1985).

	Mated ♂ n = 17	Unmated ♂ n = 18
No. infected toads	3	4
No. parasites/host	1, 2, 4	1, 1, 2, 4
Mean intensity	2.33	2.00
Relative density	0.41	0.44

Intensity: mean parasite burden amongst infected toads; relative density: mean parasite burden amongst total host population sample.

come of sexual selection. On the other hand, at the time of spawning, around 50% of males are infected by the blood-feeding monogenean *Pseudodiplorchis americanus* which they will have supported for at least a year. A range of factors characteristic of this host-parasite association suggest that it represents a good candidate for a within-species test of the Hamilton and Zuk hypothesis.

The important male secondary sexual character involved in attracting the female is vocalisation. Although the precise components of the call, by which the female discriminates among males, are not known for *S. couchii*, the process closely resembles that described by Hausfater *et al.* (1990) for *Hyla versicolor*. The activity is extremely costly energetically, and the effects are exacerbated because spadefoot toads emerge from prolonged hibernation only hours before mating begins, without replenishing depleted reserves. The parasites compete for these same reserves, and extrapolation of the measured effects of infection on fat and haematocrit should lead to a condition in which heavily-infected hosts have lower energy reserves for the demands of mate attraction. Confirmation of the extreme outcome of this pathology is provided by the laboratory experiments correlating host and parasite oviposition: hosts in the poorest condition showed no sexual activity at all.

For a proportion of females in some explosively breeding anurans, choice may be confounded by the satellite strategy of some males. The importance of this varies in different assemblies, related to population size and the operational sex ratio, and

TABLE 2. *Pseudodiploorchis americanus* population characteristics in male *Scaphiopus couchii* (mating assemblies, San Simon, Arizona; July 1986).

	Mated ♂ n = 67	Unmated ♂ n = 90
Prevalence	51%	53%
Mean intensity	5.56	5.56
Relative density	2.82	2.97
Standard deviation	4.89	4.70
Variance	23.9	22.1

it is not clear whether calling and non-calling males might exchange roles. The present study distinguishes mated males which mated successfully (by whatever means)—and therefore transmitted their genes—from those which were unsuccessful: and there was no significant difference in worm burdens in these groups. If, in this analysis, a significant number of satellites foiled female choice despite being heavily-infected, then we must conclude that satellite behaviour very effectively negates any “good-genes” model of sexual selection, including the Hamilton and Zuk hypothesis. If on the other hand the successful satellites carried lighter infections (perhaps because they are younger), then without their distorting effect, the present test would have produced the result that females select more heavily-infected males!

Observations at spawning sites in Arizona have convinced me that the impressions given by some accounts of “explosive mating” do not apply to *S. couchii*. Despite the restriction of spawning assemblies to a 7 hour timeframe, individual females seem to respond to a choice of mates with evident deliberation. Even in relatively dense assemblies, females may commonly swim in the immediate area of both calling and non-calling males for 10 min, and sometimes longer than 30 min, before heading directly to a chosen individual. The situation where females are amplexed immediately after they enter water is exceptional (Tinsley, in preparation).

Although *P. americanus* has the characteristics of a venereal infection, transmitted exclusively amongst mating hosts, it does not occur more often in successful males (see Read, 1988). Data from mating

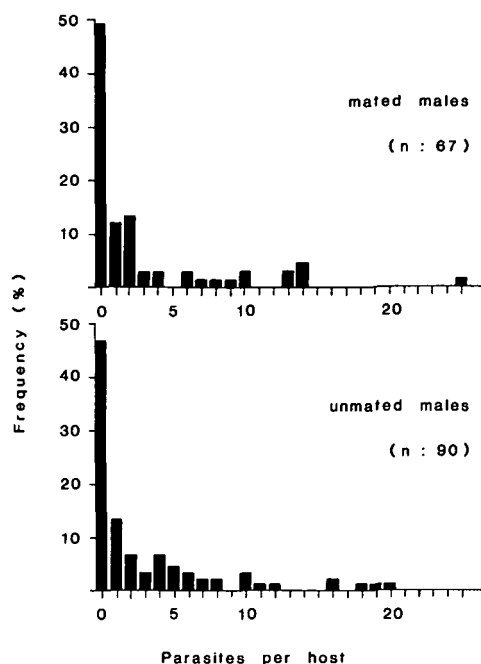


FIG. 2. Frequency distribution of infection levels of *Pseudodiploorchis americanus* in male *Scaphiopus couchii* (see Table 2).

assemblies show that every toad may become infected and, instead of infection being acquired from a sexual partner, it may matter more whether a victim floats near an infected individual—or even where that individual rested some time earlier! This mechanism of transmission, which achieves a pervasive infection, should preclude the possibility that mate choice involves a strategy of “parasite avoidance.”

Read (1988) has framed some precise requirements for the choice of systems for testing the hypothesis. The requirement that damage should be detectable at levels of infection found naturally is met by this system. However, not surprisingly, there is a strong interaction with host nutritional status which may be determined by a range of factors in addition to parasite load (see concluding discussion). This system has one important difference from those envisaged by Hamilton and Zuk (1982) and Read (1988) as ideal for the hypothesis (infections which cause heavy juvenile mortality and persist in chronic form in the survivors). *P. americanus* is a parasite only of

sexually mature hosts—which have not previously been selected, while young, for their resistance to infection. Since the period of major pathology is combined with total starvation, the impact of this parasite on a “virgin” host cohort should be even more exacting. After their abrupt initiation, all males are rigorously tested year after year for their ability to cope with or eliminate the infection. This provides a robust system for testing the hypothesis.

In many host-parasite systems, uninfected males may provide a female with ambiguous information: that state may occur by virtue of superior immune defence against a pre-existing infection, or by chance if an individual with poor immunity has never been exposed to invasion. In the *Scaphiopus*–*Pseudodiplorchis* system all males in a mating assembly become heavily-infected, and those which are parasite-free when they enter spawning sites a year later demonstrate improved fitness. The exceptions to this are the males entering mating assemblies for the first time. However, if their freedom from monogenean parasites were advantageous, then their improved mating success should be reflected in a lower mean body size among successful males compared with unsuccessful males. This is not the case (Table 3 and final discussion).

Other characteristics of the *Scaphiopus*–*Pseudodiplorchis* system also emphasise its suitability for a within-species test. Host and parasite taxa share a long co-evolutionary history, a relationship which is found in most monogenean associations. In North America, there are two subgenera (or genera) of pelobatid toads, *Scaphiopus* and *Spea*: each is infected by a different host-specific polystomatid genus, *Pseudodiplorchis* and *Neodiplorchis* respectively (Tinsley, 1981, 1983b). The separation of these host groups is considered to date from the Oligocene (Sage *et al.*, 1982) and this was probably closely paralleled by the now-distinct parasite genera.

The linking of host and parasite reproduction reinforces strict specificity (*Pseudodiplorchis americanus* infects no other host species) and produces an annual periodicity in transmission. However, because inva-

sion involves sexually-mature hosts, generation times are different: one year for the parasite but three yr for the host. Work in progress on bone growth rings (Tinsley, unpublished) shows that spadefoot toads are relatively long-lived—up to 15 yr—and this creates the possibility for co-adaptive cycles between host and parasite. During its life time, each male is challenged every year by an unavoidable infection; the parasite competes with the host for finite resources which cannot be replenished for over 80% of each year; and the ability of the host to eliminate this pathogenic parasite is tested every year. Hosts best able to achieve this completely are contained within the 50% of the male population which emerge uninfected to enter mating assemblies—and these have not suffered the extra drain on their reserves during hibernation. To the extent that the ability of these males to eliminate their infection is heritable, their success in sexual selection should, intuitively, improve population fitness and disease resistance.

Interpretation

The *Scaphiopus*–*Pseudodiplorchis* system seems eminently suitable for testing the Hamilton and Zuk model of sexual selection. However, field studies show that there is no correlation between mating success and parasite infection.

An important factor in interpreting the evolutionary consequences of this result concerns the link between host and parasite reproduction: this led me initially to seek an explanation in terms of the avirulence of the association (Tinsley, 1988). Two key factors contributed to the prediction that there should be selection against parasite pathogenicity. First, the very long period—up to 364 days/year—during which transmission is totally precluded. Clearly, if a host dies in this interval—either because of its low resistance to infection or because of the high virulence of the parasite—then the genotypes of both host and parasites will be very effectively selected against. Second, because parasite transmission is triggered by host mating activity, the toad must not only survive, it must enter the spawning assembly in peak

TABLE 3. Characteristics of male *Scaphiopus couchii* in mating assemblies (San Simon, Arizona; July 1986).

	Mated δ (n = 61)	Unmated δ (n = 71)	P
Body length (mm)	65.1	64.1	0.19
Body weight (g)	29.2	28.3	0.26
Fat weight (g)	0.25	0.26	0.69
Gonad weight (g)	0.04	0.04	0.38
Haematocrit (%)	34.5	34.5	0.85

sexual condition. I concluded therefore that a more subtle influence would also select for avirulence: any parasite which might reduce host fitness by impairing sexual behaviour would never transmit its offspring. Experimental evidence came from laboratory trials (see above) which revealed a precise synchronization between parasite oviposition and host sexual activity. In the trial involving males in very poor condition which failed to become sexually aroused, the parasites inside them failed to receive the necessary trigger and released few infective stages. If the parasites were to provoke this poor condition their transmission would be precluded. It seemed logical to deduce that this host-parasite relationship should show strong co-evolutionary trends, with the most successful parasites (which transmit their offspring) occurring in the most successful hosts (which are not disadvantaged in sexual selection) (Tinsley, 1988). However, more comprehensive data suggest that my initial conclusion requires reconsideration.

There is now good evidence of the pathological effects of *P. americanus* (see above and Tocque, 1990), reducing fat reserves first and then haematocrit. It seems reasonable to expect that in a relatively harsh desert environment, a proportion of hosts would end hibernation in poor condition (whether because of poor feeding success in the previous summer, the extra demands of infection during starvation, or the environmental conditions experienced during hibernation). Predictably, these toads could reach a state in which they would not become sexually aroused and their parasites would not receive the oviposition cue. But, the evidence based on relatively large field samples is that toads

in such poor condition simply do not occur in the mating assemblies. Hosts which do not become aroused enough to trigger parasite reproduction would not be stimulated to migrate to a breeding chorus either! For the males within an assembly, experiments have shown that the parasites receive an equally effective oviposition trigger from successful males and from those which compete unsuccessfully to secure a female. Because transmission is not linked with host gamete release (as my initial experiments led me to believe), and all infected males in a breeding assembly will release parasite progeny, there will be no selection against the parasite genotypes in unmated males.

Among males sampled from breeding assemblies, there is appreciable variation in body length and weight, fat reserves, haematocrit and parasite burden. However, there is no significant difference between mated and unmated males in any of these parameters: the overall variation is distributed equally in the two host groups (Table 3). The field data which showed a significant difference in fat weight between infected and uninfected toads (Tocque, 1990) were based on these same host population samples. However, because parasite burdens are distributed equally in mated and unmated males, the depletion of fat reserves occurs equally in both subsets of the male population. In severely-malnourished hosts, studied in the laboratory, parasite burden significantly reduced haematocrit. However, amongst a sample of over 100 males taken from these spawning assemblies, there was no significant effect of infection on haematocrit. Therefore, although infection had depleted fat in these samples, the effects of malnutrition or parasite load, or both, had not reached a threshold at which the hosts were unable to maintain essential blood composition.

Conclusions

The host-parasite system employed in this test of the Hamilton and Zuk hypothesis experiences extreme environmental conditions in a desert biome, and is subject to intense selection pressure. This begins with enormous mortality of vulnerable tad-

pole and juvenile stages. Then, every year, the adult toads are tested for their success in prey capture during the brief feeding season, for their physiological tolerance of prolonged hibernation and starvation, as well as for their ability to sustain or eliminate the extra burden imposed by parasite infection. Laboratory and field data show that *P. americanus* can significantly affect host condition and jeopardize survival (reducing reserves essential for hibernation, etc.). In my study sites, all males become infected by this pathogenic parasite, but worm burdens are then totally eliminated from about half of the toads and the others carry various levels of chronic infection. Intuitively, many animals may fail to survive hibernation from one or a combination of these factors. Others, having experienced sub-lethal effects, may survive with severely depleted reserves and high parasite burdens. To the extent that their deficiencies are genetically-determined, these males should make poor mates. My evidence suggests that hosts which survive hibernation in poor condition *are* selected against—but not by mate choice: they simply do not turn up in the spawning assemblies. The males which do form a breeding chorus have demonstrated a reasonable physiological condition and genetic fitness, in the face of harsh environmental constraints, by getting there.

Since *P. americanus* does exert a measurable influence on host condition and, by extrapolation, on host survival, I would predict that infection is a significant factor in natural selection leading to the promotion of genotypes conferring resistance to infection. However, infection is just one of several factors affecting condition, together with feeding success, tolerance of dehydration and starvation, choice of hibernation site. Indeed, the effects of these factors are undoubtedly linked: as this study shows, a host with good nutritional status does not exhibit parasite-induced pathology. Adaptation to all these influences is likely to result in improved fitness and to confer superiority as a mate. However, among the males present in a breeding assembly, from which the females actually select mates, there are no significant dif-

ferences in a range of parameters, including parasite burden: no one factor emerges from this study as being more important than anything else in influencing the condition of successful and unsuccessful males. Males which are in suboptimal condition are selected against before the process of mate choice begins.

Although this host-parasite system incorporates a range of extreme environmental factors, its application in this test of the Hamilton and Zuk hypothesis should not therefore be assessed as a special case, relevant only to explosively-breeding toads, or to desert animals, or to parasites transmitted during host reproduction. Instead, these extreme and demanding circumstances may serve very effectively to sharpen influences which are recognisable in many other mating systems. The most significant extrapolation is that selection for disease resistance may occur at a far earlier stage than the mating arena, and that although parasite-induced pathology may have an important influence on the condition of competing males it is only one of a number of vital contributory factors.

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REFERENCES

- Dimmitt, M. A. and R. Ruibal. 1980a. Environmental correlates of emergence in spadefoot toads (*Scaphiopus*). *J. Herpetol.* 14:21–29.
- Dimmitt, M. A. and R. Ruibal. 1980b. Exploitation of food resources by spadefoot toads (*Scaphiopus*). *Copeia* 1980:854–862.
- Hamilton, W. D. and M. Zuk. 1982. Heritable true fitness and bright birds: A role for parasites? *Science* 218:384–387.
- Hausfater, G., H. C. Gerhardt, and G. M. Klump. 1990. Parasites and mate choice in gray treefrogs (*Hyla versicolor*). *Amer. Zool.* 30:299–311.
- Horton, J. D. and A. M. Lackie. 1989. Evolution of immunity. In I. Roitt, J. Brostoff and D. Male (eds.), *Immunology*, 2nd ed., pp. 15.1–15.16. Gower Medical, London, New York.
- McClanahan, L. 1967. Adaptations of the spadefoot

- toad, *Scaphiopus couchi*, to desert environments. *Comp. Biochem. Physiol.* 20:73–99.
- Read, A. F. 1988. Sexual selection and the role of parasites. *Trends Ecol. Evol.* 3:97–102.
- Ruibal, R., L. Tevis, and V. Roig. 1969. The terrestrial ecology of the spadefoot toad *Scaphiopus hammondi*. *Copeia* 1969:571–584.
- Sage, R. D., E. M. Prager, and D. B. Wake. 1982. A Cretaceous divergence time between pelobatid frogs (*Pelobates* and *Scaphiopus*): Immunological studies of serum albumin. *J. Zool. (Lond.)* 198: 481–494.
- Sullivan, B. K. 1985. Sexual selection and mating system variation in pelobatids and bufonids of the Arizona-Sonoran Desert. *Great Basin Naturalist* 45:688–696.
- Taigen, T. L. and K. D. Wells. 1985. Energetics of vocalization by an anuran amphibian (*Hyla versicolor*). *J. Comp. Physiol. B* 155:163–170.
- Tinsley, R. C. 1981. The evidence from parasite relationships for the evolutionary status of *Xenopus* (Anura: Pipidae). *Monit. Zool. Ital. (NS) suppl.* 15:367–385.
- Tinsley, R. C. 1983a. Ovoviviparity in platyhelminth life cycles. *Parasitology* 86:161–196.
- Tinsley, R. C. 1983b. Ecological and phylogenetic specificity amongst polystomatid monogeneans. *Parasitology* 87(2):xi.
- Tinsley, R. C. 1988. Parasites and mating success in spadefoot toads, *Scaphiopus couchii*. *Amer. Zool.* 28: 51A. 284.
- Tinsley, R. C. 1989. Effects of host sex on transmission success. *Parasitol. Today* 5:190–196.
- Tinsley, R. C. 1990. Opportunism in parasite life cycles. In C. J. Barnard and J. Behnke (eds.), *Parasitism and host behaviour*. Taylor and Francis, London. (In press)
- Tinsley, R. C. and C. M. Earle. 1983. Invasion of vertebrate lungs by the polystomatid monogeneans *Pseudodiplorchis americanus* and *Neodiplorchis scaphiopodis*. *Parasitology* 86:501–517.
- Tinsley, R. C. and H. C. Jackson. 1986. Intestinal migration in the life cycle of *Pseudodiplorchis americanus* (Monogenea). *Parasitology* 93:451–469.
- Tinsley, R. C. and H. C. Jackson. 1988. Pulsed transmission of *Pseudodiplorchis americanus* (Monogenea) between desert hosts (*Scaphiopus couchii*). *Parasitology* 97:437–452.
- Tocque, K. 1990. *The reproductive strategy of a monogenean parasite in a desert environment*. Ph.D. Thesis, University of London.