

POLYPLOIDY AND PARASITIC INFECTION IN *XENOPUS* SPECIES FROM WESTERN UGANDA

JOSEPH A. JACKSON¹, RICHARD C. TINSLEY¹ AND STEPHEN KIGOOLO²

¹*School of Biological Sciences, University of Bristol, Bristol BS8 1UG, UK*

²*Department of Zoology, Makerere University, PO Box 7062, Kampala, Uganda*

Three *Xenopus* taxa occur with sympatric or parapatric distributions in western Uganda. Two of these, *X. wittei* and *X. vestitus*, are polyploid relative to the third, *X. laevis*, and are considered to have arisen by allopolyploidization. This is a rare mechanism of speciation by which doubling of the genome in interspecific hybrids leads to the production of a self-maintaining hybrid lineage. As one of the parental lineages of both higher polyploid species may have been related to *X. laevis*, the co-occurrence of the three taxa might show the effects of hybrid host genomes on parasite specificity. Data are reported on the natural distribution of a monogenean fluke, *Protopolystoma*, occurring in these hosts at five sites in western Uganda. It is established that *P. xenopodis*, a common parasite of *X. laevis*, is absent from wild populations of *X. vestitus* and *X. wittei* in this area. Another *Protopolystoma* species infects all three hosts. The results of other recent host-specificity studies of parasites occurring in central African *Xenopus* species are also reviewed. Observed host-parasite relationships can be related to the genealogical origin of *X. wittei* and *X. vestitus*. Hybrid organisms might inherit susceptibility to parasites from both parents and thus be exposed to infection by a greater number of species. However, the available information suggests that allopolyploid clawed toads do not show such an effect and are resistant to some potential parasites from their presumed parental lineages. The possible use of parasites as "tags" for investigating the distribution and relationships of cryptic host species is also considered.

INTRODUCTION

The pipid genus *Xenopus* includes a series of polyploid representatives, at least some of which are allopolyploid, having originated from the hybridization of separate lineages (Tymowska, 1991; Kobel, 1996). Highland lakes in western Uganda are inhabited by three taxa (Tinsley, Kobel & Fischberg, 1979). These include two octoploid species, *X. wittei* Tinsley, Kobel & Fischberg and *X. vestitus* Laurent that may have arisen from interbreeding between tetraploid ancestors related to *X. fraseri* Boulenger and *X. laevis* (Daudin) (see Tymowska, 1991). *X. wittei* and *X. vestitus* occur sympatrically with *X. l. victorinus* Ahl in western Uganda (Tinsley *et al.*, 1979): both higher polyploids thus co-occur or show adjacent distributions with each other and with a species related to one of their likely parental lineages. *X. fraseri*-like toads do not occur sympatrically with *X. wittei* or *X. vestitus* and are restricted to lowland tropical forest, a habitat which exists in the Zaire basin immediately adjacent to highland regions of the western Rift Valley occupied by *vestitus* and *wittei*. As the *Xenopus* taxa mentioned above are found within a restricted geographical area, sometimes in the same water bodies (Tinsley *et al.*, 1979), they might frequently be exposed to infective stages of the same parasite species. This is particularly likely given the great ecological similarity of clawed toads, which are all fully aquatic and feed underwater (Tinsley, Loumont & Kobel, 1996). The distribution of *Xenopus* species in western Uganda and surrounding areas therefore presents a "natural experiment" on the effects of

large scale host genetic changes on parasite specificity (Tinsley, 1981).

Protopolystoma is a polystomatid monogenean which occurs as an adult in the urinary bladder of *Xenopus* species. The life histories of monogeneans make them particularly suitable for a study of interspecific variation in host resistance to parasitism. They typically show restricted host ranges (e.g., Du Preez, Kok & Seaman, 1997; Tinsley & Jackson, 1998) and a direct life-cycle with transmission by actively swimming infective stages. The parasites' distribution is therefore unaffected by a reliance on intermediate hosts which might show varying ecological interactions with different final host species (Tinsley, 1981).

The present paper reports the distribution of *Protopolystoma* species in relatively large samples of *X. wittei*, *X. vestitus* and *X. l. victorinus* from five sites in western Uganda. Existing information on the host-specificity of these polystomatids from central African *Xenopus* (based on laboratory experiments and records of natural distributions) is summarized and data for other parasite groups are also reviewed. The significance of host ploidy level and hybrid origin on parasite distributions, and the possible uses of parasites in studies of the host group are considered.

MATERIALS AND METHODS

GENERAL

Xenopus species were collected from five localities in western Uganda (Kigezi District) in September, 1996, and immediately killed and preserved (in forma-

lin) for return to the laboratory in Bristol. Samples were taken at L. Bunyonyi (*X. wittei*, $n=63$); L. Chahafi (*X. l. victoriamus*, $n=40$); L. Echuya (*X. wittei*, $n=37$); L. Mulehe (*X. vestitus*, $n=95$; *X. wittei*, $n=6$) and L. Mutanda (*X. vestitus*, $n=74$). The urinary bladders of all toad specimens were removed and examined for the presence of *Protopolystoma* spp. Terminology for infection statistics follows Margolis, Esch, Holmes, Kuris & Schad (1982).

PARASITES

The two *Protopolystoma* species found in this study will be described or redefined elsewhere (Tinsley & Jackson, 1998). One species corresponds to the existing taxon *P. xenopodis* (Price, 1943), while the other form is referred to here as species A.

HOST IDENTITY

Present localities for *X. wittei* and *X. vestitus* are amongst those listed in the studies describing *X. wittei* and *X. kigesiensis* Tinsley (a synonym of *X. vestitus*) (see Tinsley, 1973a, Tinsley *et al.*, 1979). Where *X. wittei*-like toads originate from other sites they are termed *X. wittei* aff., as distinct morphological variants occur in some areas (R.C.T. unpublished observations). Unidentified *X. fraseri* subgroup species (see Kobel, Loumont & Tinsley, 1996) are termed *X. fraseri* aff.

RESULTS

Infection statistics for polystomatids recovered at five western Ugandan sites are presented in Table 1. Two *Protopolystoma* species were found: *Protopolystoma* species A occurred at all sites and infected all three host taxa, while *P. xenopodis* was present in *X. l. victoriamus* at L. Chahafi but absent from *X. wittei* and *X. vestitus*. Population levels of species A were lower in *X. wittei* from L. Bunyonyi and Echuya (prevalence <10%, abundance 0.1) than in *X. vestitus* from L. Mulehe and L. Mutanda (prevalence >30%, abundance 0.7-0.9). The parasite was absent from *X. wittei* in the only *vestitus/wittei* mixed sample (from L. Mulehe); however, only a small number of toads ($n=6$) was examined, so that no significance can be attached to this observation. Tinsley (1973b) reported *Protopolystoma* species A (as *P. xenopi*) in *X.*

vestitus (= *X. kigesiensis*) from L. Mutanda, based on a collection made in November, 1969. The presence of a particular *Protopolystoma* species may therefore be stable over relatively long periods of time. However, species A occurred at a lower prevalence (10%, $n=241$) and abundance (0.2) in 1969 than in 1996, indicating that its populations might show significant seasonal or longer term fluctuations. As such variations between sites could account for the higher infection levels found in *X. vestitus* from the present study, it is not possible to draw further conclusions from this feature of the data. At L. Chahafi, species A occurred at a moderate level in *X. l. victoriamus* (prevalence 18%, abundance 0.2), infecting more hosts but showing fewer worms in the overall sample than *P. xenopodis* (prevalence 10%, abundance 0.4; see Table 1).

DISCUSSION

Present results establish that *Protopolystoma xenopodis* is absent from natural populations of *X. wittei* and *X. vestitus* in western Ugandan. Both *P. xenopodis* and an undescribed species (species A) co-occurred in *X. laevis* at L. Bunyonyi, while *wittei* and *vestitus* were only infected by species A, which was present at all five sites investigated. The pattern of host-specificity revealed in this survey is consistent with data from a wider study of *Protopolystoma* biogeography (Tinsley & Jackson, 1998). This showed that species A is endemic to central Africa, having been recorded from *X. laevis* subspecies at localities in western Kenya, western Uganda, south and eastern Zaire and Rwanda (Tinsley & Jackson, 1998). It was also found in *X. wittei* and *X. vestitus* at some of the present sites (Tinsley, 1973b; Tinsley *et al.*, 1979; Tinsley & Jackson, 1998) and in *X. wittei*-like hosts from Rwanda and Burundi. *P. xenopodis*, which appears to be narrowly specific to *X. laevis*, is common in the Rift Valley region of central Africa and frequently co-occurs with Species A.

X. wittei and *X. vestitus* are octoploid forms which may have speciated as the result of allopolyploidization (Tymowska, 1991). This a mechanism by which interspecies hybrids show doubling of their genome, leading to the production of a new, self-maintaining hybrid lineage. Both species are believed to descend from tetraploids related to *X. laevis* and *X. fraseri*-like

TABLE 1. *Protopolystoma* spp. from *Xenopus* spp. in Ugandan lakes: infection statistics. (Standard deviations in parentheses.)

Locality	Host	Parasite	Prevalence	<i>n</i>	Mean intensity	Abundance
L. Chahafi	<i>laevis</i>	<i>P. xenopodis</i>	10.0%	40	4.0 (1.6)	0.4 (1.3)
		sp. A	17.5%	40	1 (0)	0.2 (0.4)
L. Bunyonyi	<i>wittei</i>	sp. A	9.5%	63	1 (0)	0.1 (0.3)
L. Echuya	<i>wittei</i>	sp. A	8.1%	37	1.3 (0.6)	0.1 (0.4)
L. Mutanda	<i>vestitus</i>	sp. A	37.8%	74	1.8 (0.9)	0.7 (1.0)
L. Mulehe	<i>vestitus</i>	sp. A	31.6%	95	2.7 (1.9)	0.9 (1.6)
	<i>wittei</i>	sp. A	0	6	0	0

toads (Tymowska, 1991). Susceptibility to *Protopolystoma* species A may therefore have been inherited by *X. vestitus* and *X. wittei*-like forms from their *X. laevis*-like parental lineage. At the same time, the absence of *P. xenopodis* from octoploid species, even though it does infect the sympatric *X. l. victorianus*, suggests that monogenean specificity may be controlled by subtle host genetic differences. Experimental studies (Tinsley & Jackson, 1998) indicate that there is a physiological barrier to the maturation of *P. xenopodis* infections in *X. wittei* from western Uganda. Apart from the two species occurring in western Ugandan clawed toads, a third representative of *Protopolystoma* was found in *X. wittei* aff. from southern Rwanda by Tinsley & Jackson (1998). This form is usually associated with *X. fraseri* aff. from lowland forest in the Zaire basin and west Africa: its occurrence in *wittei* aff. may therefore result from the genealogical relationship of this toad to a *fraseri*-like lineage.

In addition to work on *Protopolystoma* reviewed above, host specificity studies on a number of other parasite groups have recently been carried out (Tinsley 1981; Tinsley, 1996), or are in progress. Three species of the gyrodactylid monogenean *Gyrdicotylus* are found in *X. wittei*-like hosts, showing a mixture of morphological characteristics (Tinsley, 1996). One is closely related to *G. gallieni* from *X. laevis*, while the others show affinities to a species occurring in *X. fraseri* aff. This pattern may again result from the inheritance of parasite lines by the hybrid host species.

The occurrence of monogenean species in *X. wittei*-like hosts also shows geographical variation (Tinsley, 1996). Although *Protopolystoma* species A is widespread, the other species from this host was only found in southern Rwanda. Of the three *Gyrdicotylus* species mentioned above, each form occurs separately in geographically distant *X. wittei*-like populations (from western Uganda, eastern Zaire and southern Rwanda). It therefore seems possible that octoploid populations show variable susceptibilities to monogenean parasites. In the absence of experimental data confirming the resistance/susceptibility pattern of these populations it is impossible to be sure that the distributions of monogeneans are not influenced by host-independent ecological factors. In one case, however, experimental evidence suggests that the species of *Gyrdicotylus* from *X. wittei* aff. in southern Rwanda is not able to sustain population growth on type-locality *X. wittei* from western Uganda (Tinsley, 1996).

Studies of interspecific hybrid individuals have shown that levels of parasitism may be increased (Sage, Heyneman, Lim & Wilson, 1986; Dupont & Crivelli, 1988). Also, parasites from either parental host might potentially infect the hybrid offspring, so that these are exposed to larger numbers of parasite species. In the case of monogeneans, hybrid fish hosts have been found to be susceptible to parasites primarily specific to either parent species (Dupont & Crivelli, 1988; Cloutman, 1988). However, for the octoploid toads it appears that, at least at single localities, numbers of

parasite species are not increased, and some parasites from either or both related tetraploid lineages (*X. fraseri* and *X. laevis*-like) may be excluded. Apart from the example of *P. xenopodis*, this is illustrated by the distribution of the pseudophyllidean cestode *Cephalochlamys*, which occurs in all African pipids which have been examined except for *X. wittei* and *X. vestitus* (see Tinsley, 1981; also unpublished records from *X. tropicalis* and hymenochirine hosts). Such novel resistance in allopolyploid species may be the result of their hybrid genetic constitution. Immune system genes (which might be of particular importance in determining parasite specificity) can be expressed from both parental genotypes (Du Pasquier, Wilson & Robert, 1996). Genetic reorganizations subsequent to allopolyploidization have been shown to include silencing of some major histocompatibility complex genes (Du Pasquier, Miggiano, Kobel & Fischberg, 1977) and the loss of immunoglobulin genes (Du Pasquier *et al.* 1996). Hybridization might carry a penalty of increased parasitism in the short term (as seems possible from studies of other systems). However, in octoploid clawed toads, present observations suggest that either immediately, or over periods of evolutionary time, the hybrid lineages develop resistance to parasites which infected their parental species. Regional variations in *X. wittei* susceptibility to infection by monogeneans may suggest that isolated populations have undergone a differing history of the genetic rearrangements mentioned above or even originate from independent allopolyploidization events. Further experimental host specificity studies of monogenean parasites are in progress which might further clarify regional variations in the susceptibility of *X. wittei*-like toads.

Another parasite group showing clear discontinuities of distribution involving octoploid hosts is the seuratoid nematode genus, *Chitwoodchabaudia*. As the sole representative within its family (Chabaud, 1978) it does not show close relationships with any known form (Puylaert, 1970; Chabaud, 1978) and its lineage is of ancient, perhaps Mesozoic, origin (Baker, 1984). The distribution of *Chitwoodchabaudia*, which is only found in *X. wittei* and *X. vestitus*, is therefore remarkable given the relatively recent evolution of its hosts (Tymowska, 1991). This age disparity between the octoploid *Xenopus* and parasite lineages indicates that *Chitwoodchabaudia* cannot have evolved *in situ*. It must, therefore, have been inherited from an unknown parental toad species or have "jumped" from a non-*Xenopus* host.

The present results show how the study of parasite taxonomy and specificity might provide information useful in host identification. The host range of high-specificity parasites with direct life-cycles may reflect genetic differences in closely related, ecologically similar, sympatric hosts. Shared parasites may also illuminate the evolutionary relationships of their host species. For instance, the discovery of a *Chitwoodchabaudia* species amongst *X. fraseri*-like

taxa might well indicate the ancestry of *X. wittei* and *X. vestitus*. Preliminary studies of some *Protopolystoma* species have also revealed small morphological differences between samples from different hosts and locations. The variation, primarily in sclerotized structures of the attachment organ and terminal genitalia, may be genetically determined or affected by the host environment. In either case, polystomatid morphotypes might be of use in the identification of cryptic host species established by molecular methods or mating call variation (e.g. amongst the complex of *X. fraseri*-like species). Identification of parasites, which can be examined by light microscopy with minimal preparation, may be more practical than the determination of host samples by mating call or cytogenetic and molecular techniques. It is also possible to recover specimens from existing preserved museum collections of amphibians whose specific identity cannot be determined from morphological characters.

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