http://www.alliedacademies.org/parasitic-diseases-diagnosis-therapy/
ISSN: 2591-7846

PLEOMORPHISM AS A SEQUENTIAL PHENOTYPIC MANIFESTATION IN BLOOD FLAGELLATES OF DIFFERENT VERTEBRATE GROUPS

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Received: October 21, 2016; **Accepted:** October 24, 2016; **Published:** October 31, 2016

ABSTRACT

Objective: The objective of the research work was to observe pleomorphism (more than one morphological phenotypic expression of a species) in flagellate parasites (Trypanosoma and Trypanoplasma).

Methods: The blood of selected vertebrate hosts (fishes, amphibians, reptiles and mammals) was scanned for haemoflagellate infectivity by examination of fresh blood (hanging drop preparations and haematocrit) and stained smears. The flagellate parasites (Trypanosoma and Trypanoplasma) were morpho-taxonomically identified and the phenotypic manifestation expressed as pleomorphism recorded for each species.

Outcomes: Twenty-four species of trypanosomes from 12 fish hosts were discovered (12 species monomorphic, 6 each dimorphic and polymorphic). From amphibians and mammals, only monomorphic forms were encountered. The reptiles (Hemidactylus flaviviridis, Calotes versicolor) were not susceptible to trypanosome infection.

Conclusions: Host-wise analysis indicated that Heteropneustes fossilis was most susceptible to trypanosomes. Trypanoplasms from fish too exhibited pleomorphism (6 species monomorphic, 2 each dimorphic and polymorphic).

Keywords: Blood; flagellate; pleomorphism; Trypanosoma; Trypanoplasma

INTRODUCTION

Pleomorphism is the ability of organisms to alter their shape or size. It is one of the most remarkable feature of trypanosomes and is also apparent in trypanoplasms. Pleomorphism is the sequential phenotypic manifestation of a single genotype as it appears in the trypomastigote stage in the vertebrate host. The phenomenon is triggered by changes in the host antibody spectrum. There are a few fish trypanosomes which seem to be virtually monomorphic, displaying only variability in size and/or in length/width ratio. The developmental stages in their vertebrate host, if any, seem to be limited to other than morphological manifestations.

The blood flagellates, *Trypanosoma* and *Trypanoplasma* are widely reported to dwell in the blood of vertebrate hosts (Gupta, 1994., Woo, 2003., Gupta, 2006) but studies pertaining to the expression of pleomorphism are rare. The present study examines the morphology of the trypanosomes and trypanoplasms infecting different vertebrate hosts from Uttar Pradesh, India. Pleomorphism in these parasites based on intra specific morpho-variants is reported.

MATERIALS AND METHODS

Live host samples were obtained from the local fishing grounds (fish), ponds or amphibious habitats (amphibians), and domestic habitats, godowns and laboratories (lizards and rats). They were acclimatized to laboratory conditions and maintained in the Animal House of the Department of Animal Science under adequate conditions of food and water. Diagnosis of infection of blood parasites was done by hanging drop preparation or microhaematocrit (4000 rpm) and parasites observed in the buffy layer. The

parasites were identified by preparing at least 4-6 films of fresh blood on methanol cleaned slides by aseptic microtechniques. The films were air dried, fixed in methanol and stained in Giemsa/Leishman (Qualigens) and buffer (Hale, 1965) in the ratio of 1:7, mixed uniformly by a thin hair brush, stained for 40-50 mins and mounted in DPX (Gupta, 1986 and Gupta, 2010). The microphotographs were taken under oil immersion in LEICA-DMLB microscope. The forms were drawn to scale, measured, tabulated and morpho-taxonomically identified.

RESULTS

A. Trypanosome Infectivity and Pleomorphism

Flagellates discovered from fishes up to mammals could range from monomorphic to polymorphic forms. The facts given below are the compiled information of the entire work.

Twenty-four species of trypanosomes from 12 fish hosts have been discovered. Out of these, 12 species were monomorphic, 6 dimorphic and 6 polymorphic. Out of these, from *Heteropneustes fossilis*, 2 monomorphic (*T. kargenensis* Gupta and Gupta, 1994., *T. karelensis* Gupta et al. 2000), 2 dimorphic (*T. artii* Gupta et al. 2002., *T. heteropneusti* Gupta et al. 2006) and one polymorphic (*T. singhii* Gupta and Jairajpuri, 1981) species were discovered. From *Channa punctatus*, one monomorphic (*T. rohilkhandae* Gupta and Saraswat, 1991), 2 dimorphic (*T. aligaricus* Gupta and Jairajpuri, 1982., *T. saulii* Gupta et al. 2006) and one polymorphic (*T. bareilliana* Gupta et al. 1987) were discovered. From *Colisa* (*Trichogaster*) *fasciata*, 2 monomorphic (*T. trichogasteri* Gupta and Jairajpuri, 1981., *T. piscidium* Gupta et al. 2003), one new variety (*T. trichogasteri* var. nov. *fasciatae*) and one polymorphic (*T. colisi* Gupta, 1986) species were discovered. From *Mystus vittatus*, one monomorphic (*T. bagroides* Gupta and Gupta, 1996) and one dimorphic (*T. tengari* n.sp., Gupta et al. 2015) whereas from *Catla catla*, one monomorphic (*T. monomorpha* Gupta and Jairajpuri, 1985) and one new variety (*T. monomorpha* var. nov. *catlae* Gupta et al., 2000) were discovered. Amongst the remaining species, 3 of them were monomorphic (*T. notopteri* Gupta and Jairajpuri, 1985 from *Notopterus notopterus*; *T. ritae* Gupta and Yadav, 1989 from *Rita rita*; *T. danilewskyi* Laveran and Mesnil, 1904 from *Cyprinus carpio*), one dimorphic (*T. batrachi* Gupta and Jairajpuri, 1983 from *Clarias batrachus*) and three polymorphic (*T. attii* Gupta and Jairajpuri, 1981 from *Wallago attu*; *T. barbi* Gupta et al. 1987 from *Barbus barbus; T. ticti* Gupta et al. 1998 from *Puntius ticto*) species have been put on record (Table 1).

In addition to the above, representative vertebrates from Amphibia (*Rana tigrina, Bufo melanostictus*), Reptilia (*Hemidactylus flaviviridis, Calotes versicolor*), Aves (*Columba livia*) and Mammals (*Rattus rattus* from India and *Clethrionomys glareolus*, *Apodemus flavicollis* and *Microtus oceanus* from Poland) were also examined for flagellate parasites. Amongst these, the infected hosts were *Bufo melanostictus* (*T. bisalpurensis* n. sp. unpublished), *Rana tigrina* (*T. rotatorium* Mayer, 1843), *Rattus rattus* [*Trypanosoma (Herpetosoma) lewisi* Laveran and Mesnil, 1901], *Clethrionomys glareolus* [*Trypanosoma* (*Herpetosoma*) *evotomys* Molyneux, 1969], *Apodemus flavicollis* [*Trypanosoma* (*Herpetosoma*) *grosi kosewiense* Karbowiak and Wita, 2004] and *Microtus oeconomus* [*Trypanosoma* (*Herpetosoma*) *microti* Laveran and Pettitt, 1909] (Table 1). It was interesting to conclude that amongst fishes, monomorphic, dimorphic as well as polymorphic forms were observed but from amphibians and mammals, only monomorphic forms were encountered. The reptiles (*Hemidactylus flaviviridis, Calotes versicolor*), however, were not prone to trypanosome infection. Host-wise analysis indicated that *Heteropneustes fossilis* was most susceptible to trypanosomes (Table 2).

B. Trypanoplasm Infectivity and Pleomorphism

Trypanoplasms are characteristic of fishes. Ten species of this parasite have been recorded during this entire period. These parasites too exhibited pleomorphism: 6 forms being monomorphic, 2 dimorphic and 2 polymorphic. *Mystus vittatus* was infected with *T. guptii* Gupta and Gupta, 1987 (polymorphic) and *T. tengari* Gupta et al. 1988 (monomorphic), *Cyprinus carpio* with *T. golysiana* Gupta and Pilarczyk, 1994 (monomorphic) and *T. polygolysiana* Gupta and Pilarczyk, 1994 (polymorphic); *Clarias batrachus* with *T. (Cryptobia) maguri* Gupta and Gupta, 1997 (dimorphic), *T. haematalis* Gupta and Gupta, 2010 (monomorphic) and *T. monomorpha.* n.sp (unpublished) (monomorphic). *Puntius ticto* was infected with *T. cyprinoides* Gupta et al. 1998 (dimorphic) and a new variety, *T. sarnae* var. nov. *sophorae* Gupta and Gupta, 2014 and *Catla catla* with *T. catli* Gupta et al. 1999 (monomorphic) (Table 3). Host-wise analysis for trypanoplasm infectivity indicated that *Clarias batrachus* was the most prone species (Table 4).

DISCUSSION

It has been observed in the past that during the course of infection, most fish trypanosomes display forms successively differing conspicuously in their shape and size; they were designated by early authors (Laveran, 1912) as the pleomorphic forms, "*var. parva* " or "*var. magna* " and sometimes these forms were erroneously credited with separate taxonomic status (Fantham, 1919), as even reported in later times (Bauer, 1984). However, it soon became clear that these are but extremes in the sequence of

Table 1
List of *Trypanosoma* Species.

S.No.	Parasites	Hosts	Forms	Authors
Fish Hosts				
1.	*T. trichogasteri*	*Trichogaster fasciata*	Monomorphic	Gupta and Jairajpuri, 1981
2.	*T. singhii*	*Heteropneustes fossilis*	Polymorphic	Gupta and Jairajpuri, 1981
3.	*T. attii*	*Wallago attu*	Polymorphic	Gupta and Jairajpuri, 1981
4.	*T. aligaricus*	*Ophiocephalus punctatus*	Dimorphic	Gupta and Jairajpuri, 1982
5.	*T. batrachi*	*Clarias batrachus*	Dimorphic	Gupta and Jairajpuri, 1983
6.	*T. monomorpha*	*Catla catla*	Monomorphic	Gupta and Jairajpuri, 1985
7.	*T. notopteri*	*Notopterus notopterus*	Monomorphic	Gupta and Jairajpuri, 1985
8.	*T. colisi*	*Colisa fasciata*	Polymorphic	Gupta, 1986
9.	*T. bareilliana*	*Channa punctatus*	Polymorphic	Gupta et al.1987
10.	*T. barbi*	*Barbus barbus*	Polymorphic	Gupta et al.1987
11.	*T. ritae*	*Rita rita*	Monomorphic	Gupta and Yadav, 1989
12.	*T. rohilkhandae*	*Channa punctatus*	Monomorphic	Gupta and Saraswat, 1991
13.	*T. kargenensis*	*Heteropneustes fossilis*	Monomorphic	Gupta and Gupta, 1994
14.	*T. danilewskyi* Laveran and Mesnil, 1904	*Cyprinus carpio*	Monomorphic	Gupta and Pilarczyk, 1994
15.	*T. bagroides*	*Mystus vittatus*	Monomorphic	Gupta and Gupta, 1996
16.	*T. trichogasteri* var. nov. *fasciatae*	*Colisa fasciatus*	Monomorphic	Gupta et al. 1998
17.	*T. ticti* n.sp.	*Puntius ticto*	Polymorphic	Gupta et al. 1998
18.	*T. monomorpha* var. nov. *catlae*	*Catla catla*	Monomorphic	Gupta et al. 2000
19.	*T. karelensis*	*Heteropneustes fossilis*	Monomorphic	Gupta et al. 2000
20.	*T. artii*	*Heteropneustes fossilis*	Dimorphic	Gupta et al. 2002
21.	*T. piscidium*	*Colisa fasciata*	Monomorphic	Gupta et al. 2003
22.	*T. saulii*	*Channa punctatus*	Dimorphic	Gupta et al. 2006
23.	*T. heteropneusti*	*Heteropneustes fossilis*	Dimorphic	Gupta et al. 2006
24.	*T. tengari*	*Mystus vittatus*	Dimorphic	Gupta et al. 2015
Amphibian hosts				
1.	*T. bisalpurensis* n.sp.	*Bufo melanostictus*	Monomorphic	Unpublished
2.	*T. rotatorium*	*Rana tigrina*	Monomorphic	Mayer, 1843
Mammalian hosts				
1.	*Trypanosoma* (*Herpetosoma*) *lewisi*	*Rattus rattus*	Monomorphic	Laveran and Mesnil, 1901
2.	*Trypanosoma* (*Herpetosoma*) *evotomys*	*Clethrionomys glareolus*	Monomorphic	Molyneux, 1969
3.	*Trypanosoma* (*Herpetosoma*) *grosi kosewiense*	*Apodemus flavicollis*	Monomorphic	Karbowiak and Wita, 2004
4.	*Trypanosoma* (*Herpetosoma*) *microti*	*Microtus oeconomus*	Monomorphic	Laveranand Pettitt, 1909

forms occurring during the course of infection with one species (Minchin, 1909., Briendl, 1915., Laird, 1951., Khan, 1976). Out of the large number of piscine *Trypanosomes*, only in a few has the full sequence been established.

In such pleomorphic species, the first trypanosomes to appear in the blood of the infected host are the “young” forms-rather small and slender with a few, shallow waves of the undulating membrane and nucleus revealing no distinct karyosome. After some time, intermediate forms appear until, eventually, “adult” forms prevail to persist during the chronic phase of infection. They are large, stout, sometimes very wide, and usually have a richly spiraling undulating membrane with numerous bends, a nucleus with a prominent karyosome and sub-surface striation. They often have more chromatic granules in their cytoplasm and free flagellum is shorter than in the young forms. Depending on the phase of infection at which the fish is examined, one finds young, slender or large adult forms only, or intermediate ones. A mixture of forms is probably the result of sequential leech feeds.

Table 2
Host-Wise List of Trypanosomes Discovered

Hosts	Parasites	Forms	Authors
Fish Hosts			
Heteropneustes fossilis	*T. singhii*	Polymorphic	Gupta and Jairajpuri, 1981
	T. kargenensis	Monomorphic	Gupta and Gupta, 1994
	T. karelensis	Monomorphic	Gupta et al. 2000
	T. artii	Dimorphic	Gupta et al. 2002
	T. heteropneusti	Dimorphic	Gupta et al. 2006
Channa punctatus	*T. aligaricus*	Dimorphic	Gupta and Jairajpuri, 1982
	T. bareilliana	Polymorphic	Gupta et al. 1987
	T. rohilkhandae	Monomorphic	Gupta and Saraswat, 1991
	T. saulii	Dimorphic	Gupta et al. 2006
Colisa fasciata	*T. trichogasteri*	Monomorphic	Gupta and Jairajpuri, 1981
	T. colisi	Polymorphic	Gupta, 1986
	T. trichogasteri var. nov. fasciatae	Monomorphic	Gupta et al. 1998
	T. piscidium	Monomorphic	Gupta et al. 2003
Mystus vittatus	*T. bagroides*	Monomorphic	Gupta and Gupta, 1996
	T. tengari	Dimorphic	Gupta et al. 2015
Catla catla	*T. monomorpha*	Monomorphic	Gupta and Jairajpuri, 1985
	T. monomorpha var. nov. catlae	Monomorphic	Gupta et al. 2000
Wallago attu	*T. attii*	Polymorphic	Gupta and Jairajpuri, 1981
Clarias batrachus	*T. batrachi*	Dimorphic	Gupta and Jairajpuri, 1983
Notopterus notopterus	*T. notopteri*	Monomorphic	Gupta and Jairajpuri, 1985
Barbus barbus	*T. barbi*	Polymorphic	Gupta et al. 1987
Rita rita	*T. ritae*	Monomorphic	Gupta and Yadav, 1989
Cyprinus carpio	*T. danilewskyi* Laveran and Mesnil, 1904	Monomorphic	Gupta and Pilarczyk, 1994
Puntius ticto	*T. ticti*	Polymorphic	Gupta et al. 1998
Amphibian hosts			
Bufo melanostictus	*T. bisalpurensis* n. sp.	Monomorphic	Unpublished
Rana tigrina	*T. rotatorium*	Monomorphic	Mayer, 1843
Mammalian hosts			
Rattus rattus	*Trypanosoma* (*Herpetosoma*) *lewisi*	Monomorphic	Laveran and Mesnil, 1901
Clethrionomys glareolus	*Trypanosoma* (*Herpetosoma*) *evotomys*	Monomorphic	Molyneux, 1969
Apodemus flavicollis	*Trypanosoma* (*Herpetosoma*) *grosi kosewiense*	Monomorphic	Karbowiak and Wita, 2004
Microtus oeconomus	*Trypanosoma* (*Herpetosoma*) *microti*	Monomorphic	Laveran and Pettitt, 1909.

The existence of a sequence of different morphological types of trypanoplasms during the course of infection in carps was firmly established early (Keysselitz, 1906). Although he misinterpreted numerous stages both in the blood and in the leech as ‘gametes’, his reproduction of polymorphism is the most complete to be found in existing reports. The sequence of stages was confirmed in goldfish (Robertson, 1911), in loaches (Briendl, 1915) and in tench (Kipp, 1968) trypanoplasms. *Trypanoplasma catostomi* in parasite-free fingerlings of the host fish were noted (Bower, 1977) and the authors observed a remarkable polymorphism including the size as well as differing body structure coupled with the progress of the infection originally started by the introduction of a single flagellate into the host. In view of the paucity of information, sequential pleomorphism in blood-stream stages of trypanoplasms may or may not be a generally distributed phenomenon (Lom, 1979).

Table 3
List of *Trypanoplasma* Discovered from Fish Hosts

S.No.	Parasites	Hosts	Forms	Authors
1.	*T. guptii*	*Mystus vittatus*	Polymorphic	Gupta and Gupta, 1987
2.	*T. tengari*	*Mystus vittatus*	Monomorphic	Gupta et al. 1988
3.	*T. golysiana*	*Cyprinus carpio*	Monomorphic	Gupta and Pilarczyk, 1994
4.	*T. polygolysiana*	*Cyprinus carpio*	Polymorphic	Gupta and Pilarczyk, 1994
5.	*T.* (*Cryptobia*) *maguri*	*Clarias batrachus*	Dimorphic	Gupta and Gupta, 1997
6.	*T. cyprinoides*	*Puntius ticto*	Dimorphic	Gupta et al. 1998
7.	*T. catli*	*Catla catla*	Monomorphic	Gupta et al. 1999
8.	*T. haematalis*	*Clarias batrachus*	Monomorphic	Gupta and Gupta, 2010
9.	*T. monomorpha.* n.sp.	*Clarias batrachus*	Monomorphic	Unpublished
10.	*T. sarnae* var. nov. *sophorae*	*Puntius ticto*	Monomorphic	Gupta and Gupta, 2014

Table 4
Host-Wise List of Trypanoplasms Discovered from Fish Hosts

S.No.	Hosts	Parasistes	Forms	Authors
1.	*Mystus vittatus*	*T. guptii*	Polymorphic	Gupta and Gupta, 1987
2.		*T. tengari*	Monomorphic	Gupta et al. 1988
3.	*Cyprinus carpio*	*T. golysiana*	Monomorphic	Gupta and Pilarczyk, 1994
4.		*T. polygolysiana*	Polymorphic	Gupta and Pilarczyk, 1994
5.	*Clarias batrachus*	*T.* (*Cryptobia*) *maguri*	Dimorphic	Gupta and Gupta, 1997
6.		*T. haematalis*	Monomorphic	Gupta and Gupta, 2010
7.		*T. monomorpha.* n.sp.	Monomorphic	Unpublished
8.	*Puntius ticto*	*T. cyprinoides*	Dimorphic	Gupta et al. 1998
9.	*Catla catla*	*T. catli*	Monomorphic	Gupta et al. 1999
10.	*Puntius ticto*	*T. sarnae* var. nov. *sophorae*	Monomorphic	Gupta et al. 2014

Pleomorphism is expressed in the following features:

1. Size changes.
2. Changes in length/width ratio i.e. slender or broad forms.
3. The number, width and depth of the waves of the undulating membrane.
4. The presence or absence of a distinct karyosome in the nucleus by light microscopy.
5. Presence and number of stainable cytoplasmic granules.
6. Length of the free end of the flagellum.
7. Distance of the kinetoplast from the posterior end.
8. Shifts in the position of the nucleus in the body.
9. Presence of subsurface pellicular striation ("myonemes") in stained preparations by light microscopy (longitudinal or spirally oriented striations, probably sub-pellicularly located ribbons of mitochondrial systems.

Separate morphological stages may be defined by the combination of the above characters; marked changes of points 1 to 5 are proper to truly polymorphic species while changes in points 1, 2, 6, 7, 8, 9 may also be found in "monomorphic" species.

Pleomorphism in *Trypanosoma* is well marked in having a markedly variegated shape and five different stages in varying combinations at various periods of the digenetic life cycle (vertebrate and invertebrate hosts) may occur (trypomastigote, amastigote, promastigote, sphaeromastigote and metacyclic stages). However, trypomastigotes are the predominant stages reported from fish blood, but in the vertebrate blood too, pleomorphism may be visible as seen above.

Pleomorphism in fish trypanosomes has been reported by some authors (Becker 1979 Joshi 1979) without assigning any names whereas others have given specific names to the various forms. 'Large' and 'small' forms of *T. remarki* (Laveran, 1907); Type I, II and III (Tanabe, 1925); small, medium and large (Dutton 1907); small and large forms of *T. striati* (Qadri, 1955)*;* large and

stumpy forms of *T. batrachi* (Qadri, 1962); slender and broad forms of *T. occidentalis* (Becker, 1967); short, elongated and stumpy forms of *T. aori* (Joshi, 1982) have been distinguished in different species of *Trypanosoma* by various authors.

The form that predominates depends on the course of infection, which again in its turn is subject to many factors. Although this time sequence of morphs has been verified in some species only (e.g. *T. barbatulae* Breindl, 1915., *T. murmanensis* Khan, 1976), it most probably holds true for all of the polymorphic species. The most marked examples of pleomorphism can be drawn from among marine species, e.g. those from skates-*T. giganteum, T. rajae, T. gargantua.* The freshwater examples are *T. granulosum, T. percae and T. remarki.*

In fish blood flagellates, a condition similar to trypanosomatids of other host groups exists. Pleomorphism is presumed to be sequential and is regarded to be a phenotypic expression of the genotype (Lumsden, 1976). However, as aptly stated (Bardsley, 1973), only observation of the parasite in its vertebrate host when it is subjected to a complete variety of naturally-occurring conditions and factors can reveal whether it really is monomorphic. In experimental transfers among various hosts (carps, goldfish, gudgeon, pike, *Barbus conchonius*), varying temperature regimes or study of various stages of infection failed to reveal any true pleomorphism (Lom, 1979).

Bloodstream forms of the initial stages of infection are always rather small and of a regular shape (young stages have comma-like or crescent-like shapes). In more advanced infections, bigger, very irregular forms prevail, reflecting in their shape wave-like undulatory movements of the whole body. In the chronic, often long lasting, period of infection, the number of trypanoplasms was reduced; assuming a large size and an irregular shape, sometimes with numerous large bends of the undulating membrane; there was a nucleus with a conspicuous endosome not seen in young stages (Lom, 1979). The length of flagellum relative to the body length may also greatly vary during the cycle, as well as the number of stainable granules in the body.

Given the extreme metaboly of the cell shape, it is difficult to characterize the shape differences in young and advanced infection stages in more precise terms but they are quite conspicuous as are the differences in size. For example, in *Trypanoplasma* sp. from pikes (*T. guerneyorum*), the average size of a young form is 20 X 4 µm (excluding flagella), while in the chronic stage form it is 38 × 9 µm.

The large forms of trypanosomes and trypanoplasms found in late infection in the circulating blood were considered to be "adult" forms and were conveniently used as reference stages for taxonomic comparisons (Lom, 1979). While it seems certain that *Trypanosoma murmanensis* is competent to initiate growth in the vector (Khan, 1978), there is no evidence as to whether it is this or some cryptic, tissue-inhabiting, persisting, stage which is responsible for relapses of parasitaemia.

CONCLUSION

In this report we describe the morphological polymorphism of trypanosomes and trypanoplasms, which includes the different trypomastigote phenotypes from the blood of different vertebrates of Uttar Pradesh, India.

The survey indicates that pleomorphism amongst fish hosts appears to be a regular feature. However, amongst amphibian and mammalian hosts, monomorphism was dominant suggestive of chronic trypanosomiasis. But even in fish hosts, it is safe to consider that monomorphism dominates (12 species) as compared to dimorphism (6 species) and polymorphism (6 species) as visualized above.

ACKNOWLEDGEMENT

Thanks are expressed to the Uttar Pradesh Council of Agricultural Research (UPCAR), Lucknow for providing financial assistance by awarding a major research project.

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