Writing – some "benefits" of experience?

John Janovy, Jr.

With the help of numerous editors, critics, and literary agents, some employed as such and others not.

Quotes to start the day:

- *"Please don't embarrass the family by trying to publish this."* – Cindy Janovy
- *"An evocative book about ideas, exactly the kind of thing the American book-buying public is getting increasingly impatient with."* – Miriam Gooderich
- *"John, I can't get into your new book."* – Karen Janovy

More quotes to start the day:

- *"Particularly offensive."* – J. F. Mueller
- *"Why do you waste your postage sending us things that don't turn us on?"* – some forgotten editor
- *"I don't know what this book is about."* – Jane Dystel

(There are probably more; these are the ones that come immediately to mind.)

Some simple rules and guiding philosophies:

- It’s not your soul or the measure of your worth; it’s double-spaced typing.
- Editors are not always right.
- Some critics are never right.
- Literary agents are not always right.
- If you really try to do something well, you’ll usually screw it up and never finish.

Some more simple rules and guiding philosophies:

- Plan your narrative line and devices in advance.
- Make your paragraphs 1/3 to ½ page long.
- Put your pictures and tables in order first.
- Write the results section first.
- Write the methods section next.
- Write the discussion next and the introduction last.

Now, some insider stuff:

46, 30, 16, 10, 18, 18, 8 – see SI

This advice is well above and beyond what is necessary to outwit profs who assign papers, but I'm inclined to pass it along anyway just because some of you might be unusual students, not necessarily unusually *good* students, but just unusual in some ill-defined way. My final piece of advice is to practice writing, especially if you are at an institution in which profs tend to assign a lot of papers or give essay exams. Write; about anything—personal letters, opinions, stuff just to get something off your chest but would never send. But write in complete sentences—subject, verb, object, prepositional phrases. Something in one sentence needs to remind a reader of what has been said in the previous sentence. About two or three times a page, your string of sentences ought to bring some idea to closure. Then you're ready to start a new paragraph.

Now, some insider stuff:

12, 30, 31, 28, 22

Practice paragraphing, deciding when there is a natural break between sentences. The first sentence of one paragraph should remind a reader of what has been said in the previous paragraph, although each paragraph should be a piece of "stand alone" literature. In other words, if you lift that paragraph out of your paper and print it on a blank sheet, then all by itself it should tell a small and complete story. This requirement means that the opening sentence needs to establish the subject of that paragraph, and the closing sentence ought to finish a short discussion of that subject. A reader who finishes the paragraph ought to think "that makes sense" and thus be prepared for your next idea or assertion.

Now, some insider stuff: [16]

The above two paragraphs are quite self-referential in the sense that they illustrate this advice.

There is no evidence in this archival set of drawings that such design was the handiwork of a supernatural intelligence, or that the artist would have even considered that possibility.

We do have evidence, however, that his pencil habits were probably formed early because they were so completely ingrained. Cleaning out what remains of John Janovy's drawing equipment, more than thirty years after his death, I find dozens of pencils, the wood shaved, the lead exposed and filed to a needle point, in all kinds of colors and degrees of hardness. I envision him shaving and sharpening these pencils, carefully, exactly, over a wastebasket, all by himself, alone in an office, no telephone, no radio, no noise whatsoever except the muffled sound of traffic seven floors below, to distract a petroleum geologist from his preparation of an instrument to draw a line on a piece of paper, a line that reveals how convergence of natural processes leads to the production of those magic resources that fuel tanks, sending them in a cloud of dust across some desert terrain, or self-propelled 155mm howitzers dug in, lined up, covered with camouflage netting, recoiling with their own internal explosions, or P-51 Mustangs screaming across the sky, or jeeps straight out of some Bill Mauldin cartoon. With these finely sharpened pencils, he draws the lines that point to oil.

30, 19, 42, 121, 13

Some examples of stuff:

ADVISORY TO READERS

Today's Life, etc. section includes a photo of a famous fresco by Michelangelo that includes nudity.

(Front page notice in the Raleigh, NC *News and Observer*.)

(From ARTnews, November, 2006)

ADVISORY TO READERS

Today's *Life, etc.* section has a photograph of a famous Sistine Chapel fresco by Michelangelo. The original fresco, completed in 1486, has Adam depicted as nude.

JJ's edit

Reprinted from the JOURNAL OF PARASITOLOGY
Vol. 63, No. 5, October 1977
Made in United States of America

HERPETOMONAS MEGASELIAE AND *CRITHIDIA HARMOSA*: GROWTH ON BLOOD-AGAR PLATES

Amy Doran Keppel and J. Janovy, Jr.
School of Life Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68588

ABSTRACT: A technique for culturing two species of trypanosomatid flagellates on blood-agar plates is described. *Herpetomonas megaseliae* and *Crithidia harmosa* were grown as discrete clone colonies on plates prepared in sterile petri dishes with a medium derived from that of Tobie et al. (1950, J Parasitol **36**: 48–54). Plates were inoculated with 0.01 ml flagellate suspension and the inoculum was spread evenly over the surface of the agar with a sterile glass spreader. Plates were incubated in a high humidity chamber at 25 C and colonies of both species, easily visible to the unaided eye, appeared within 1 week. There was a virtual 1:1 ratio between calculated number of CFU (colony forming units) and actual CFU observed on the plates. Colony morphology differed between the two species. *Herpetomonas megaseliae* produced a translucent flattened colony with a raised center and raised spiral arms, while *C. harmosa* produced an opaque hemispherical colony. Applications of the culture technique include potential diagnosis of trypanosomatid infections and potential studies in trypanosomatid genetics.

Agar plates have been used for many years for the culture of microorganisms, and agar-based media have been used for nearly as long for culture of protozoa not normally grown on plates (Nöller, 1917). Modifications of plating techniques have enabled workers to study the genetics of not only the cultured microorganisms, but also their parasites (Franklin et al., 1965; Lederberg and Lederberg, 1953; Puck, 1959). In the present paper we extend the technique of agar plate culture to include two protozoan species of the family Trypanosomatidae and suggest possible applications of the technique to problem areas in the study of trypanosomatid biology.

MATERIALS AND METHODS

Laboratory stock cultures of *Herpetomonas megaseliae* Daggett, Dollahon, and Janovy 1972 (ATCC # 30209) and *Crithidia harmosa* McGhee, Hanson, and Schmittner 1969, were used. The *C. harmosa* stock was obtained from Dr. S. H. Hutner, Pace University, and is from the same stock as ATCC # 30256. Organisms were loop transferred to 5-ml Mansour's medium with human blood (Dollahon and Janovy, 1971) and incubated at 25 C for 72 hr before use. Cultures used in these experiments were in the exponential phase of growth.

Culture medium was prepared from the blood-agar base of that of Tobie et al. (1950) with the Locke's solution overlay salts added directly to the agar. Medium composition was thus: 10 g Bacto agar (Difco), 1.5 g Bacto beef (Difco), 2.5 g Neopeptone (Difco), 4 g NaCl, 0.1 g $CaCl_2$, 0.1 g KCl, 0.15 g NaH_2PO_4, 1.25 g glucose, and 500 ml distilled water. This solution was boiled and adjusted to pH 7.2, autoclaved in a 1 liter screw cap flask, and allowed to cool until the flask was just warm to the touch. Forty ml of outdated whole human blood, lysed and diluted 1:1 with distilled water, was added per 200 ml medium.

Blood-agar plates were prepared in sterile glass petri dishes (15 by 100 mm) poured with 20 ml complete medium and allowed to solidify. Stock cultures of *C. harmosa* and *H. megaseliae* were counted by hemocytometer and diluted to contain the desired theoretical number of colony forming units (CFU) per 0.01 ml dilution medium. The dilution medium was Mansour's base (Dollahon and Janovy, 1971). Plates were then inoculated with 0.01 ml flagellate suspension and Giemsa-stained slides were also made of 0.01 ml inoculum suspension. The droplet on the agar plate was spread with a sterile Pasteur pipet with the tip bent at a right angle and sealed in a flame.

Plates were sealed with masking tape, labeled, and placed in a high humidity chamber at 25 C. After 5 days growth, the numbers of colonies were counted and checked against the theoretical number of CFU inoculated. Inoculum slides were checked for differentiation state (of *H. megaseliae*) according to the methods of Janovy et al. (1975).

The high humidity chamber consisted of plastic shoe boxes (10 by 15 by 30 cm) or aquaria (16 by 16 by 20 cm) with covers, containing several cotton balls soaked in water. All inoculation procedures were carried out in an isolation hood sterilized by 24-hr continuous UV lighting.

Smears of organisms were air dried, fixed in absolute methanol, and Giemsa-stained. Stain was diluted 1:50 with phosphate buffer at pH 7.2.

RESULTS

Initial attempts to culture *C. harmosa* on agar plates as described were uniformly success-

Received for publication 14 December 1976.

Now, some science - - -

Agar plates have been used for many years for the culture of microorganisms, and agar-based media have been used for nearly as long for culture of protozoa not normally grown on plates (Nöller, 1917). Modifications of plating techniques have enabled workers to study the genetics of not only the cultured microorganisms, but also their parasites (Franklin et al., 1965; Lederberg and Lederberg, 1953; Puck, 1959). In the present paper we extend the technique of agar plate culture to include two protozoan species of the family Trypanosomatidae and suggest possible applications of the technique to problem areas in the study of trypanosomatid biology.

This narrative technique is actually borrowed from the legal profession. You don't leave a "juror" any room for doubting what should be common knowledge, i.e., a given, that establishes context.

From the introduction – a variation on the "now, we know that blah blah blah . . . " theme.

DISCUSSION

The major contributions of this paper are (1) the demonstration that trypanosomatid flagellates can be grown as discrete colonies, interpreted as clones, on blood-agar plates, and (2) the demonstration that colony morphology on the same culture medium differs in the two genera and species studied. Thus two species of the protozoan family Trypanosomatidae, viz. *Herpetomonas megaseliae* and *Crithidia harmosa*, can be studied experimentally using techniques similar to those which have been employed for over 20 years in the study of *Trichomonas vaginalis* and for much longer in the study of bacteria (Asami et al., 1955). In addition, the observed differences in colony morphology can be attributed at least to the generic differences between the two species, and thus may have a genetic basis.

Although agar plate cultures of *Trichomonas* species have been used for many years, both for cloning and diagnosis (Asami et al., 1955;

an
assa
(Jar
abo
in t
tinc
T
gra
the
236

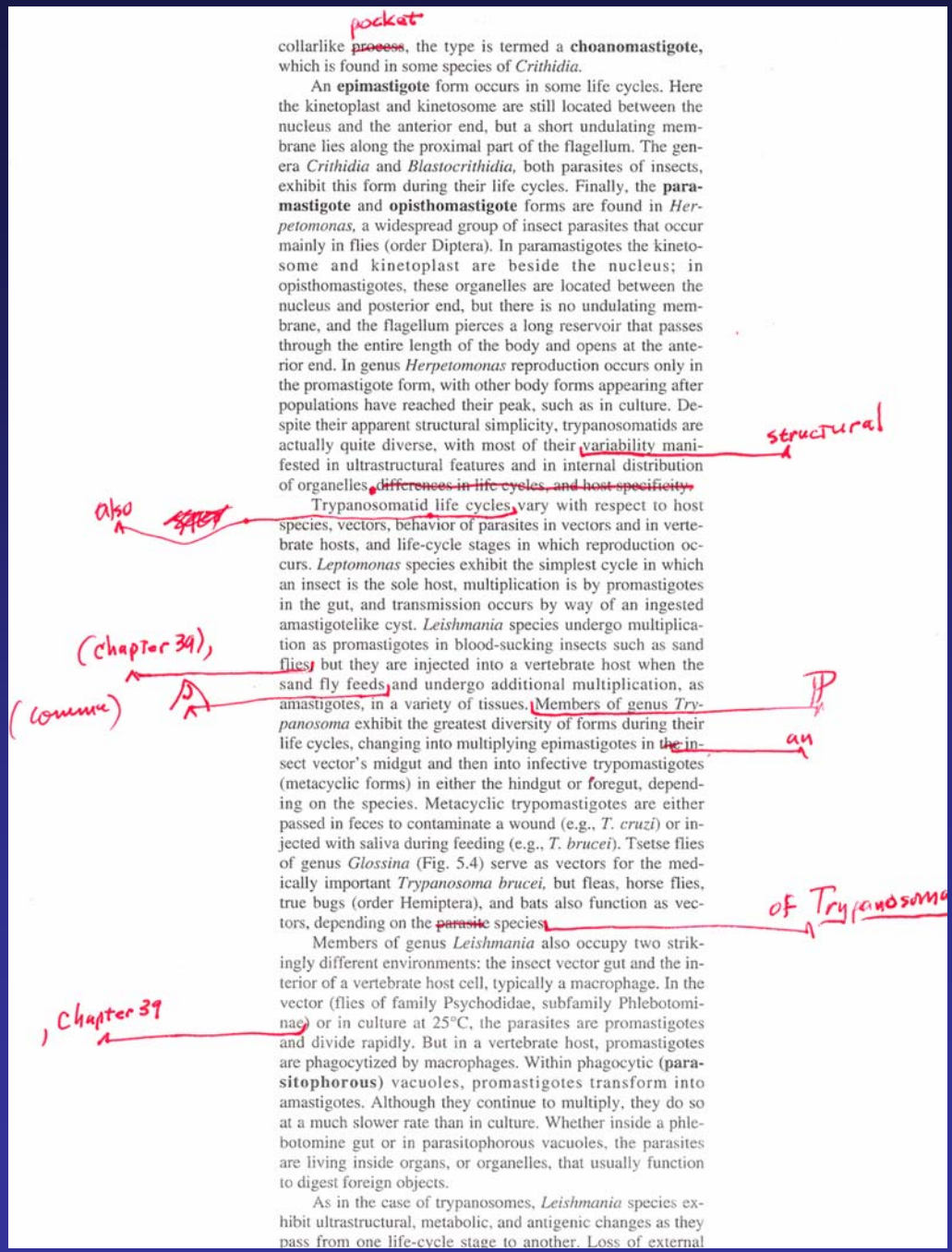
Helping the reviewers understand what you've accomplished – a media technique, as well as a seminar technique. Note also the logical train of thought (demonstration, demonstration, thus, similar, can be attributed.)

CONCURRENT INFECTIONS AND THE COMMUNITY ECOLOGY OF HELMINTH PARASITES

John Janovy Jr.,
School of Biological Sciences, University of Nebraska–Lincoln, Lincoln, Nebraska 68588-0118. *email: jjanovy1@unl.edu*

In the early 1960s, John Holmes published 3 papers from his doctoral dissertation research, started at Rice University under the incisive supervision of Asa Chandler and completed under the equally incisive, if somewhat ornery, eyes of Clark P. Read. The first of these papers (Holmes, 1961; reprinted herein), established conclusively that 2 species of parasites interacted with one another in their common environment, such interaction being prerequisite to application of the "community" concept. This paper thus marks the beginning of modern community ecology as applied to parasitic helminths. The second paper (Holmes, 1962a) further defined the nature of interactions between *Hymenolepis diminuta* and *Moniliformis dubius* (= *moniliformis*). But if Holmes (1961, 1962a) established helminth community ecology as a rich, legitimate, and useful subdiscipline of parasitology, then Holmes (1962b) cast an intriguing shadow over his own results, for the 2 parasites did not interact with one another in the hamster as they did in the rat. Thus, the 1962 papers told us that the secrets of symbiont interspecific

A novelist's technique – start with a simple description of an act, or a setting, to establish the context and the boundary conditions within which your narrative will take place.



collarlike process, the type is termed a **choanomastigote,** which is found in some species of *Crithidia.*

An **epimastigote** form occurs in some life cycles. Here the kinetoplast and kinetosome are still located between the nucleus and the anterior end, but a short undulating membrane lies along the proximal part of the flagellum. The genera *Crithidia* and *Blastocrithidia,* both parasites of insects, exhibit this form during their life cycles. Finally, the **paramastigote** and **opisthomastigote** forms are found in *Herpetomonas,* a widespread group of insect parasites that occur mainly in flies (order Diptera). In paramastigotes the kinetosome and kinetoplast are beside the nucleus; in opisthomastigotes, these organelles are located between the nucleus and posterior end, but there is no undulating membrane, and the flagellum pierces a long reservoir that passes through the entire length of the body and opens at the anterior end. In genus *Herpetomonas* reproduction occurs only in the promastigote form, with other body forms appearing after populations have reached their peak, such as in culture. Despite their apparent structural simplicity, trypanosomatids are actually quite diverse, with most of their variability manifested in ultrastructural features and in internal distribution of organelles, differences in life cycles, and host specificity.

Trypanosomatid life cycles vary with respect to host species, vectors, behavior of parasites in vectors and in vertebrate hosts, and life-cycle stages in which reproduction occurs. *Leptomonas* species exhibit the simplest cycle in which an insect is the sole host, multiplication is by promastigotes in the gut, and transmission occurs by way of an ingested amastigotelike cyst. *Leishmania* species undergo multiplication as promastigotes in blood-sucking insects such as sand flies, but they are injected into a vertebrate host when the sand fly feeds, and undergo additional multiplication, as amastigotes, in a variety of tissues. Members of genus *Trypanosoma* exhibit the greatest diversity of forms during their life cycles, changing into multiplying epimastigotes in the insect vector's midgut and then into infective trypomastigotes (metacyclic forms) in either the hindgut or foregut, depending on the species. Metacyclic trypomastigotes are either passed in feces to contaminate a wound (e.g., *T. cruzi*) or injected with saliva during feeding (e.g., *T. brucei*). Tsetse flies of genus *Glossina* (Fig. 5.4) serve as vectors for the medically important *Trypanosoma brucei,* but fleas, horse flies, true bugs (order Hemiptera), and bats also function as vectors, depending on the parasite species.

Members of genus *Leishmania* also occupy two strikingly different environments: the insect vector gut and the interior of a vertebrate host cell, typically a macrophage. In the vector (flies of family Psychodidae, subfamily Phlebotominae) or in culture at 25°C, the parasites are promastigotes and divide rapidly. But in a vertebrate host, promastigotes are phagocytized by macrophages. Within phagocytic (**parasitophorous**) vacuoles, promastigotes transform into amastigotes. Although they continue to multiply, they do so at a much slower rate than in culture. Whether inside a phlebotomine gut or in parasitophorous vacuoles, the parasites are living inside organs, or organelles, that usually function to digest foreign objects.

As in the case of trypanosomes, *Leishmania* species exhibit ultrastructural, metabolic, and antigenic changes as they pass from one life-cycle stage to another. Loss of external

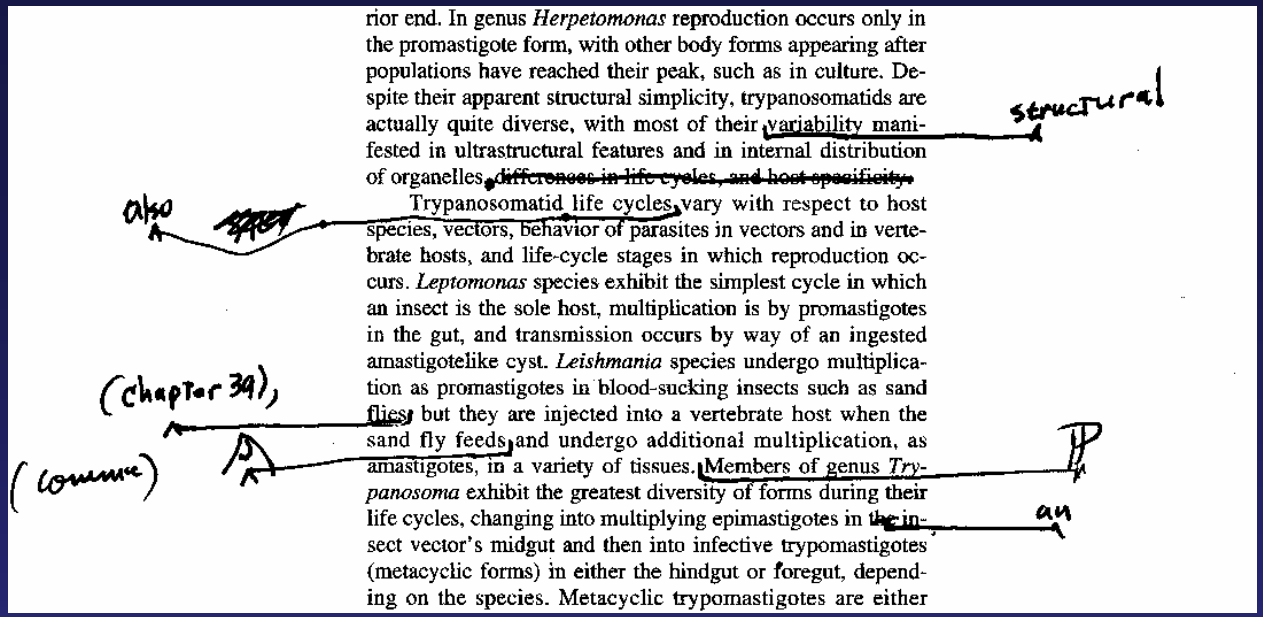
Edits from the current and upcoming edition of *Foundations of Parasitology*

(a page out of chapter 5)

rior end. In genus *Herpetomonas* reproduction occurs only in the promastigote form, with other body forms appearing after populations have reached their peak, such as in culture. Despite their apparent structural simplicity, trypanosomatids are actually quite diverse, with most of their variability manifested in ultrastructural features and in internal distribution of organelles, ~~differences in life cycles, and host specificity.~~

Trypanosomatid life cycles vary with respect to host species, vectors, behavior of parasites in vectors and in vertebrate hosts, and life-cycle stages in which reproduction occurs. *Leptomonas* species exhibit the simplest cycle in which an insect is the sole host, multiplication is by promastigotes in the gut, and transmission occurs by way of an ingested amastigotelike cyst. *Leishmania* species undergo multiplication as promastigotes in blood-sucking insects such as sand flies, but they are injected into a vertebrate host when the sand fly feeds, and undergo additional multiplication, as amastigotes, in a variety of tissues. Members of genus *Trypanosoma* exhibit the greatest diversity of forms during their life cycles, changing into multiplying epimastigotes in the insect vector's midgut and then into infective trypomastigotes (metacyclic forms) in either the hindgut or foregut, depending on the species. Metacyclic trypomastigotes are either

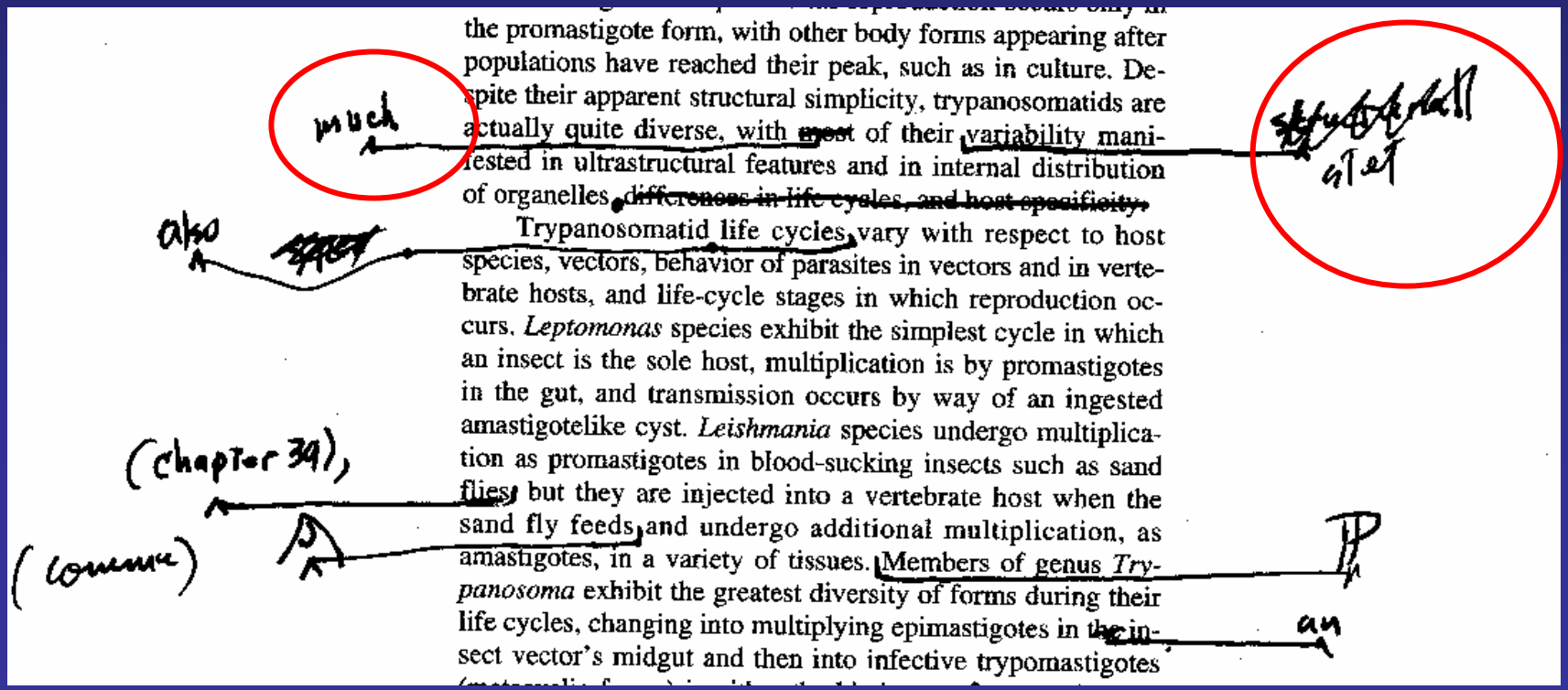
This editing is actually an attempt to reduce reading level (but note the hackneyed usage of "structural").



rior end. In genus *Herpetomonas* reproduction occurs only in the promastigote form, with other body forms appearing after populations have reached their peak, such as in culture. Despite their apparent structural simplicity, trypanosomatids are actually quite diverse, with most of their variability manifested in ultrastructural features and in internal distribution of organelles, ~~differences in life cycles, and host specificity.~~

Trypanosomatid life cycles vary with respect to host species, vectors, behavior of parasites in vectors and in vertebrate hosts, and life-cycle stages in which reproduction occurs. *Leptomonas* species exhibit the simplest cycle in which an insect is the sole host, multiplication is by promastigotes in the gut, and transmission occurs by way of an ingested amastigotelike cyst. *Leishmania* species undergo multiplication as promastigotes in blood-sucking insects such as sand flies, but they are injected into a vertebrate host when the sand fly feeds, and undergo additional multiplication, as amastigotes, in a variety of tissues. Members of genus *Trypanosoma* exhibit the greatest diversity of forms during their life cycles, changing into multiplying epimastigotes in the insect vector's midgut and then into infective trypomastigotes (metacyclic forms) in either the hindgut or foregut, depending on the species. Metacyclic trypomastigotes are either

"Structural" is hackneyed.



the promastigote form, with other body forms appearing after populations have reached their peak, such as in culture. Despite their apparent structural simplicity, trypanosomatids are actually quite diverse, with most of their variability manifested in ultrastructural features and in internal distribution of organelles, ~~differences in life cycles, and host specificity.~~

Trypanosomatid life cycles vary with respect to host species, vectors, behavior of parasites in vectors and in vertebrate hosts, and life-cycle stages in which reproduction occurs. *Leptomonas* species exhibit the simplest cycle in which an insect is the sole host, multiplication is by promastigotes in the gut, and transmission occurs by way of an ingested amastigotelike cyst. *Leishmania* species undergo multiplication as promastigotes in blood-sucking insects such as sand flies, but they are injected into a vertebrate host when the sand fly feeds, and undergo additional multiplication, as amastigotes, in a variety of tissues. Members of genus *Trypanosoma* exhibit the greatest diversity of forms during their life cycles, changing into multiplying epimastigotes in the insect vector's midgut and then into infective trypomastigotes

Animal parasites are often characterized as having complex life cycles, meaning a series of discrete ontogenetic stages, each evidently requiring a particular set of environmental conditions. In such life cycles, a sequence of environments thus allows development from egg to adult, although usually a particular stage encounters its requisite environment by chance. Ecological factors typically contribute to the probability of this event, for example as when intermediate and definitive hosts frequent the same habitat, although certain parasite attributes can increase the probability of encounter, a good example being trematode cercarial behavior that puts infective stages in a favorable microhabitat (Fingerhut et al., 2003; Haas, 2003). Parasite complex life cycles are thus systems in which survival of individuals occurs in several different, but of necessity sequential, contexts that can be quite variable but nevertheless are also characterized regionally, at least within broad limits. For example, whatever combination of aquatic habitats, temperature, and invertebrate communities allows a particular species of frog lung fluke to exist and be distributed among its various host populations in North Carolina, that combination is not likely to be replicated in New Mexico (Dronen, 1977; Wetzel, 1996). Because of such local and regional variability, the evolutionary forces acting on such ontogenetic cycles can be quite difficult to predict, or even to speculate upon.

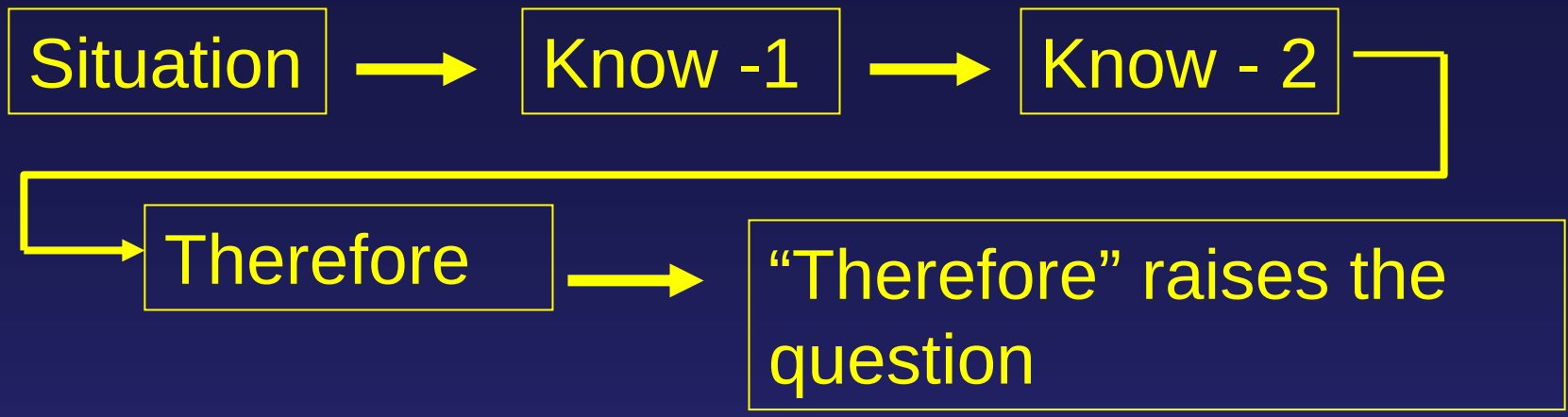
Needs a transition

26, 26, 53, 37, 47, 26

Trematodes (Cl. Trematoda: Ph. Platyhelminthes) are the classic example of parasite complex life cycles (Fig. 1), as well as the group perhaps most amenable to study of their life cycle evolution. Such amenability stems from a variety of reasons, but most importantly because trematode stages are well defined structurally, the sequence of ontogenetic steps is tightly bound to a sequence of hosts or host types, and each stage has its unique ecological characteristics (Roberts and Janovy, 2005). To date, there has been a substantial and reasonably successful effort to discover and describe parasite complex life cycles and a less substantial, but nevertheless somewhat successful, effort to discover factors driving the evolution of such cycles (e.g., Sukhdeo and Sukhdeo, 2004; Lefebvre and Poulin, 2005). Numerous authors have proposed models for the origin of complex life cycles, especially with regard to the addition or loss of intermediate hosts (Pearson, 1972; Gibson, 1981; O'Grady, 1985; Brown et al., 2001; Parker et al., 2003), but there is far less in the literature, in terms of either empirical observations or theory, regarding evolutionary processes at work on these cycles once they were established. Nevertheless, intriguing attempts have been made to explore the evolutionary contributions of virulence and larval reproduction (Davies et al., 2001), transmission mechanisms (Criscione and Blouin, 2004), and a generalist strategy at the second intermediate host stage (Rauch and Kalbe, 2005). What's missing from all these evolutionary studies is a discussion of the "right" questions to ask and means for developing hypotheses to address these questions. This paper is one attempt to sort through all the potential problems that could be studied in order to find the ones that truly influence complex life cycle evolution.

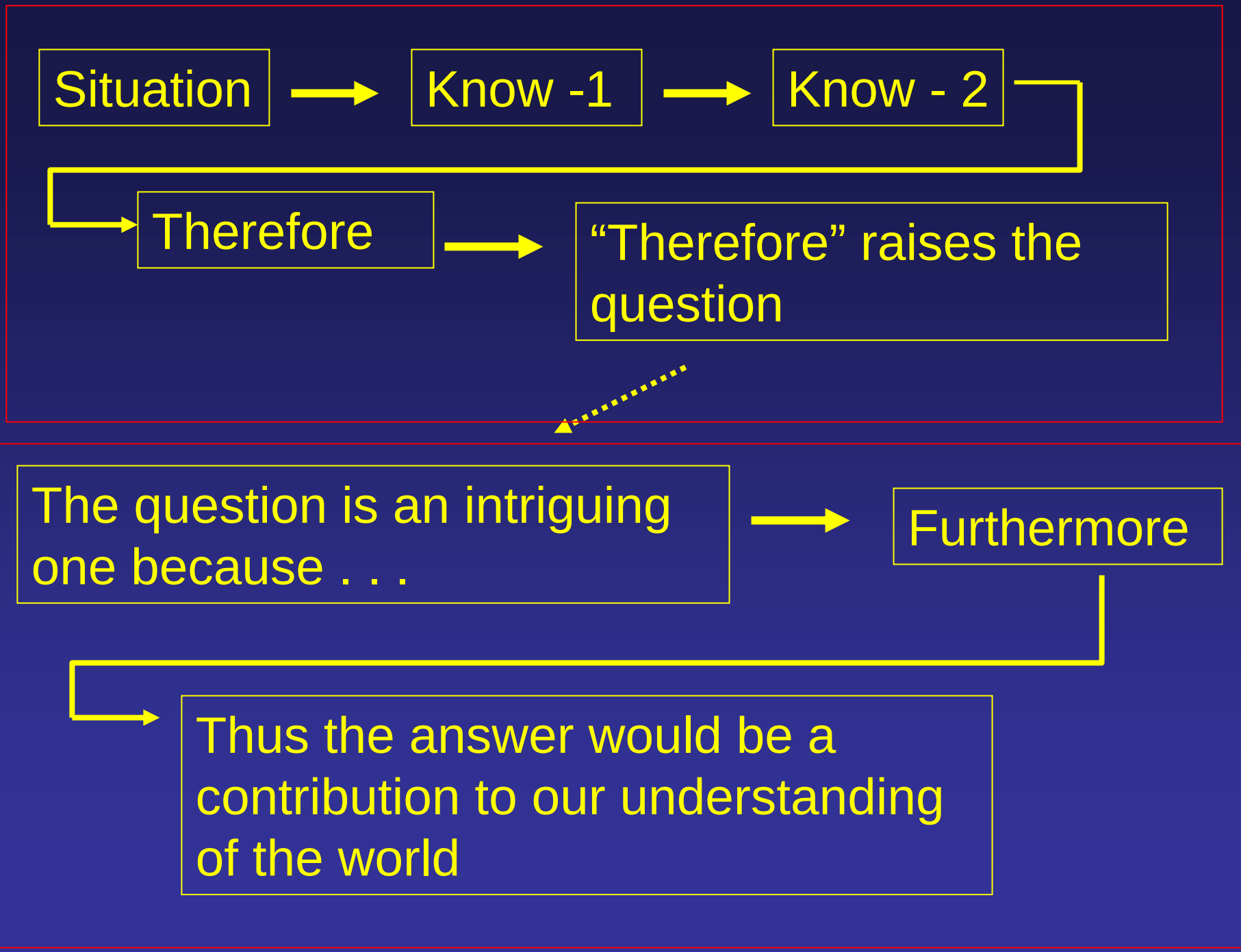
Take home messages:

- Focus initially on your sequence of points and the transitions.


Situation
Know -1
Know - 2
Therefore
“Therefore” raises the question

Take home messages:

- Focus initially on your sequence of points and the transitions.
- Next look at your paragraphs, especially their transitions and “self-contained” nature.

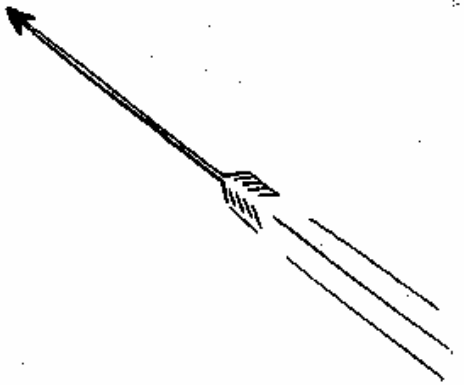

Situation
Know -1
Know - 2
Therefore
“Therefore” raises the question
The question is an intriguing one because . . .
Furthermore
Thus the answer would be a contribution to our understanding of the world

Take home messages:

- Focus initially on your sequence of points and the transitions.
- Next look at your paragraphs, especially their transitions and “self-contained” nature.
- Practice reading your stuff in different observational modes (grammar mode, spelling mode, reading level mode, indefinite reference mode, etc.)

Take home messages:

- Focus initially on your sequence of points and the transitions.
- Next look at your paragraphs, especially their transitions and “self-contained” nature.
- Practice reading your stuff in different observational modes (grammar mode, spelling mode, reading level mode, indefinite reference mode, etc.)
- Send it off.



THE ARROW AND THE SONG

I SHOT an arrow into the air,
It fell to earth, I knew not where;
For, so swiftly it flew, the sight
Could not follow it in its flight.

I breathed a song into the air,
It fell to earth, I knew not where;
For who has sight so keen and strong,
That it can follow the flight of song?

Long, long afterward, in an oak
I found the arrow, still unbroke;
And the song, from beginning to end,
I found again in the heart of a friend.

HENRY WADSWORTH LONGFELLOW

It's just double spaced typing on a page, folks.