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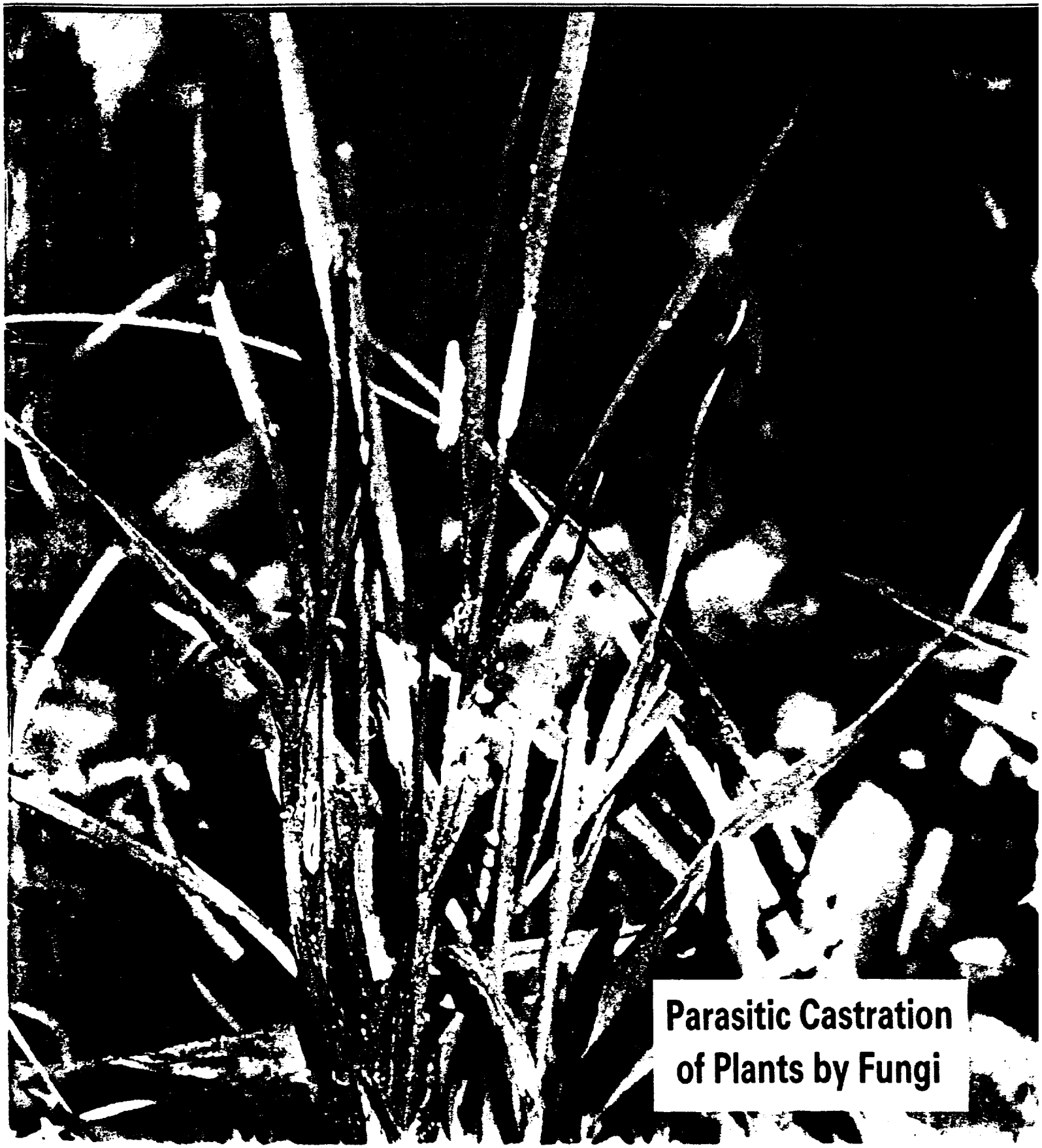

Parasitic Castration of Plants by Fungi

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Ontoecogenophyloconstraints? The Chaos of Constraint Terminology

Janis Antonovics and Peter H. van Tienderen

The world of evolutionary biology today is being bombarded with all kinds of possible constraints to the process of natural selection. Are we witnessing the end of the neodarwinistic theory of evolution, as some may like to see it, or is it just another whim of giving new names to old things? Here, we attempt to unravel the meaning and name-giving of constraints in a small and nonrandom sample of the literature, and suggest a way out from the present confusion of usages.

Most biologists probably never did believe that evolution always leads to the best of all possible worlds, but they have been so terrorized[1] into championing the inadequacies and limitations of the evolutionary process that it has become fashionable and almost *de rigueur* to invoke constraints as a central theme in evolutionary explanations[2,3]. As microevolutionists, threatened by the prospect that the neodarwinian forces of which we are so fond might just be gossamer in a tempest of larger macroevolutionary processes, we decided to explore whether the concept of constraints did indeed provide new answers and new directions.

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However, our lab discussions soon became bogged down in problems of definitions and conceptual confusion, and eventually ended in abject consternation. Are all constraints developmental or genetic in origin? Can they be both? How can we measure constraints, and what then are the units for the different kinds of constraints? Some argued that every constraint was genetic, others adamantly advocated the inviolate nature of physical laws and the constraints they engender, while a minority had no patience for what seemed to be a hopeless exercise in semantics.

We were on the verge of abandoning the topic altogether when we decided to investigate explicitly how the term 'constraint' was used in the literature. Each of us more or less haphazardly chose a paper that used the word and then traced the context that led the authors to invoke the presence of a constraint and what led them to use a particular adjective in describing that constraint.

After our initial survey of this literature, it was soon evident that the term constraint appeared in widely different settings. A number of papers dealt with constraints on abundance and distribution of species. While ultimately there might be an evolutionary explanation for this, these papers were clearly ecologically oriented and we excluded them from further scrutiny. It was then clear that most of the remaining papers dealt with evolutionary constraints to adaptation by natural selection, and the presence or absence of variation and covariation in particular traits. Natural selection requires the presence of phenotypic variation and differential fitnesses among phenotypes; for evolutionary response, the variation has to have a heritable component. Using these three premises as a guideline, the papers could be classified according to whether the traits that were studied varied phenotypically or not, and whether this variation had a genetic basis.

Names and more names

The results of our survey revealed an enormous range of adjectives applied to constraints. There were not only developmental[4] and phylogenetic[5] constraints, but also cytogeometric[6], morphological[7], physiological[8], ecological[9,10], pleiotropic[11], environmental[12] and mechanical[13] constraints; even more labels surfaced in our peripheral reading. The adjectives were sometimes applied to the trait being discussed[7], sometimes to the level of variation[5], sometimes to a process responsible for the constraint[11]. Authors would even change the adjective used to describe the constraint either in successive papers of their own (e.g. from morphological to anatomical[7,14]), or in reference to the work of others (e.g. from developmental to pleiotropic[11,15]).

Box 1. A model null model

In <taxonomic group> the evolution of <trait(s)> from their ancestral <state(s)> has/has not occurred, because phenotypic variation was <absent/present>, and the selective forces, <observed/inferred on the basis of ... >, were of a <direction/magnitude> <insufficient/sufficient> to produce the derived traits.

If the magnitude/direction was sufficient but observed response was different from expected, then the selection response was directed by

(1) genetic (co)variances of ancestral <traits>, which in turn were determined by:
- the spectrum of available mutations/recombinations
- pleiotropy/linkage disequilibrium among traits that are synchronous/sequential in development

(2) population structure, for example by:
- small population size
- gene flow
- selection acting at a different (e.g. non-individual) level

(3) coevolutionary responses of associated species.

In our small sample, examples of each of these usages could be found and there seemed no predominant or favored pattern. We took cold comfort in the realization that the confusion we felt in our earlier discussions may be general to the discipline as a whole. The papers, covering a variety of traits, usually dealt with the absence of (genetic or phenotypic) variation, or with particular associations between different traits or between a trait and an environmental factor. The level of observation could be variation within a population or variation among widely separated taxonomic groups such as marsupial and other mammalian carnivores. In most studies, the fitness differential associated with the traits was taken for granted, or was assumed from indirect sources. Mythical constraints are the expected bedfellows of mythical forces of selection!

Mechanisms and null models

The most difficult part was understanding the mechanism of the constraint. We agree with Gould[16] that a distinction needs to be made between a 'favored theory' and factors external to that theory: it is those factors that influence the process but are external to the favored theory that should be termed constraints. However, we prefer the term 'null model' to favored theory, because the latter not only implies something rather grandiose, but is also prejudicial and implicitly suggests that the favored theory is inadequate.

A constraint seems to have little meaning without a specific reference to a null model. For instance, physiological trade-offs may determine the optimal pattern of allocation to different functions[8]. Within this null model, selection may freely change the frequency of genes affecting allocation. In this sense, evolution is unconstrained, as evolution can mould allocation patterns. However, evolution is constrained in the sense that it is assumed that the trade-off is inevitable. As soon as a new mutant arises that increases the acquisition of resources, this constraint (temporarily) disappears, until the mutant becomes fixed[17].

The overall null model used by most authors was one of evolution by natural selection (irrespective of the level of selection). The alternative major null model could have been one of evolution by genetic drift, and indeed one of the papers[18] discussed the low rate of base-pair substitution in the functional region of a gene as an evolutionary constraint. This low rate was postulated to come about as a result of selection for invariant amino-acid sequences in the functional domain of an enzyme. Given evolution by random drift as a null model, natural selection now becomes a constraint!

Where is the way out?

We could see no ready-made solution to the present terminological confusion. It was obvious, however, that the simple use of an adjective to describe a constraint is completely unhelpful. Even when the authors may be clear on the context and usage of their adjective, readers may have a completely different interpretation. We therefore strongly urge that authors should state the null model explicitly; usually this null model describes what phenotypic variation to expect given a set of assumptions on the presence and expression of genetic variation. In the case where the overall null model is one of evolution by natural selection (as opposed to, say, random fixation of neutral mutations), the logical sequence displayed in Box 1 should be incorporated into any argument pertaining to constraints.

Corresponding logical arguments could be applied to cases where the null model is one of evolution by other mechanisms, such as fixation of neutral mutations, or speciation by founder effects. It is clear that information on all components of the selective explanation displayed in Box 1 may not be available[19]. For instance, if one is dealing with fossil data, selection is often inferred from functional considerations, and the lack of phenotypic variation is often inferred from the consistent absence of such variants in closely related taxa but its presence in more widely related groups. The nature of the incompleteness and the assumptions should be stated wherever there is incomplete information on the components of the evolutionary process.

A clear exposition of the null model obviates altogether the need to use the term constraint. Superficially, this may threaten to remove a paper from the fashionable mainstream of current evolutionary biology, but a temporary moratorium on constraint usage may contribute to clearer thinking within the field.

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Letter to the Editor

Animal Tissue Collections for Molecular Genetics and Systematics

Population biologists and ecologists have a responsibility to preserve the molecules of life of animals that are sacrificed during surveys, especially of those taxa whose systematics or general biology are poorly known. When planning a collecting trip to remote places, scientists should think carefully about how they will save the molecular information, as well as the morphological or physiological data.

Recent technical advances in DNA studies for genetics and systematics are rendering tissue collections more and more valuable for the study of animal phylogeny. Sequencing of mitochondrial DNA after DNA amplification by the polymerase chain reaction (PCR) can be achieved successfully using minute amounts of tissues from different sources, such as isolated hairs, feathers, pieces of dried skin, or small pieces of ethanol-preserved tissues[1]. Also, it is now easy to extract and purify moderately long chains (around 10 000 to 100 000 base pairs) of nuclear DNA from animal tissues properly preserved in 95% (or even 75%) ethanol. DNA extracted from alcohol-preserved tissues can be cloned, or amplified by PCR, or even used for solution DNA–DNA hybridization experiments[2].

Although the best way to preserve animal tissues for molecular studies in genetics and systematics is deep freezing (after an initial step in the field involving liquid nitrogen or dry ice), frozen tissue collections are expensive to maintain and to build up[3]. Ethanol preservation of tissues, on the other hand, requires only the ethanol and some leakproof, unbreakable vials. For an excellent preservation of DNA, one must try, as soon as possible after the death of the animal, to cut the tissue into pieces the size of rice grains and to immerse them in at least ten times their volume of 95% ethanol (75% ethanol is also satisfactory). After a few (two to ten) hours, the 'old' fluid is removed and replaced by fresh 75% (or 95%) ethanol, and the preserved tissues can be placed in a small labelled vial. Good-quality scintillation vials, which hold about 25 ml, are perfect. Well-preserved samples do not need to be stored at cold temperatures, and can be mailed in regular small parcels. See Ref. 4 for an excellent discussion of the collection and storage of tissues.

Tissue samples should be labelled and documented in the same way as regular museum specimens, i.e. with the species name, date and locality of capture, collector's name and field number. Field books of collectors should mention that tissues have been preserved.

A tissue collection has great scientific value if a few conditions are met, such as the proper documentation of each sample, the existence of updated files listing the preserved taxa and indicating whether other scientists can 'borrow' material from the collection. Scientists should deposit the tissue samples they have preserved in natural history museums that develop and maintain tissue collections. Institutions such as laboratories of zoology, of wildlife biology, of animal ecology, etc., should be strongly encouraged to develop permanent collections of well-preserved tissues, provided they make them available to the scientific community. (There is at least one curatorial problem unique to tissue collections[4]: the materials stored here are usually consumed as they are analysed by borrowers. A tissue 'loan' therefore diminishes the available sample, and an efficient inventory system is necessary for management of the collection.)

The collection of ethanol-preserved tissues maintained at the Laboratory of Paleontology of the Institut des Sciences de l'Evolution at Montpellier consists of about 800 samples of mammalian tissues, spanning about 300 species and representing about 170 genera of 55 families. Most samples come from a single wild individual, but some are the result of pooling two to five animals of the same geographic population. The taxa that are the best represented in our collection are some genera of Soricidae (*Sorex, Crocidura*), Arvicolidae (*Microtus*) and Muridae (*Apodemus, Mus*), and the 'families' Muridae, Gerbillidae and Sigmodontinae. Rodents make up 70% of the taxa at the species level, followed by Insectivora and Artiodactyla. Most tissue samples can be related to individual specimens, thus enabling morphological and molecular investigations on the same animals. The good state of preservation is maintained by renewing the preserving fluid (75% or, since 1989, 95% ethanol) once a year, and sizing gels of extracted DNAs are routinely performed to check the quality of the nucleic acids.

Our policy for sending tissue material (or even purified DNAs) to scientists outside the laboratory is to ask for details of the research project of the applicant, and to request that the repository and the name of the original collector are properly acknowledged in any publication resulting from their use.

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