PROVIDENCE MACHINES

#4

WARMING 2004

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borderline fealty obfuscate paranoiac agate additional drudgery hinge many eng meadowsweet homecome ike engel bootstrapping
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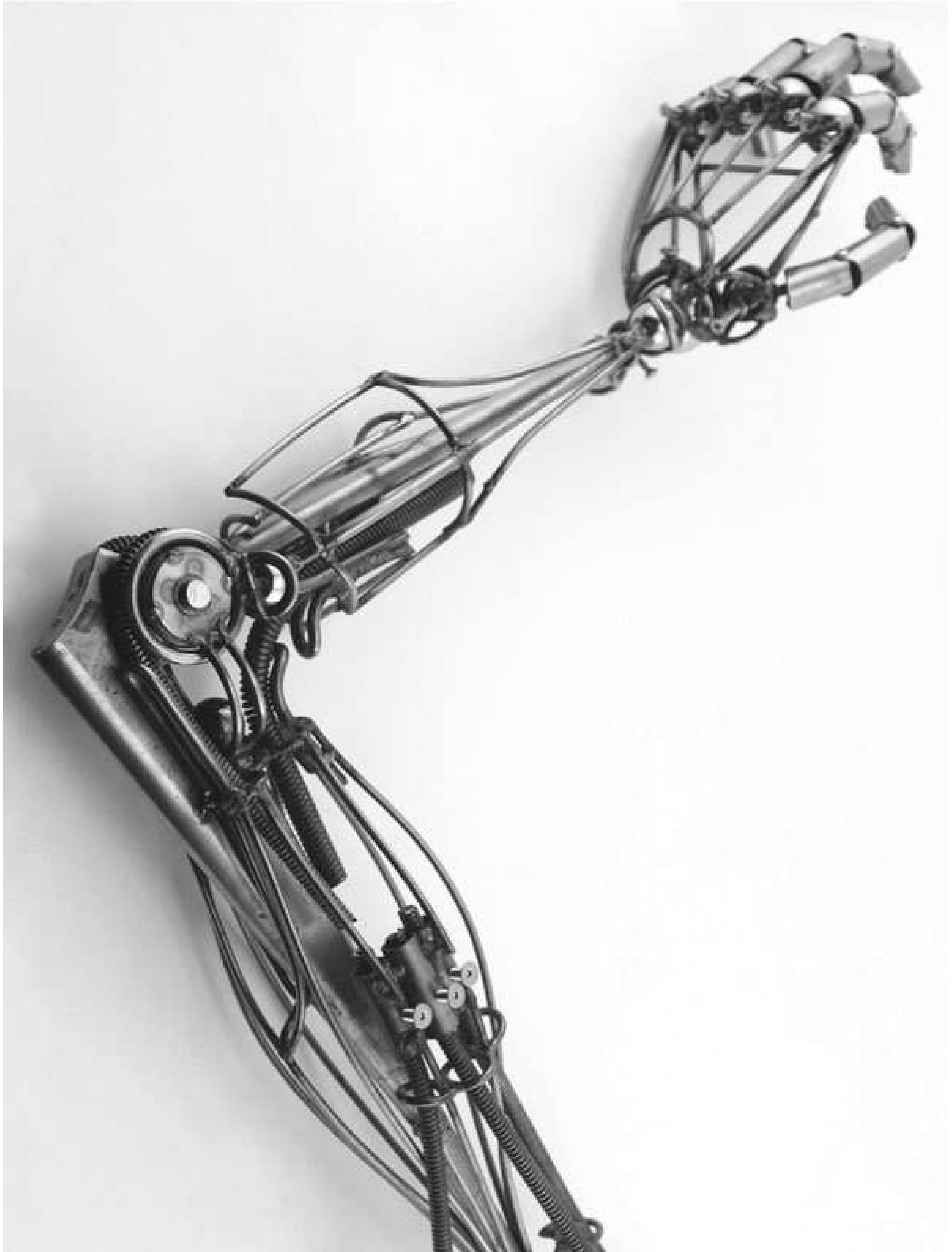


The atomic girl Five Series combat guardian
(Closeup of arm.) Greg Brotherton

```
/*
* Switch to stack of the new process and set up
* his segmentation registers.
*/
retu(rp->p_addr);

/*
* If the new process paused because it was
* swapped out, set the stack level to the last call
* to savu(u_ssav).  This means that the return
* which is executed immediately after the call to aretu
* actually returns from the last routine which did
* the savu.
*
* You are not expected to understand this.
*/
if(rp->p_flag&SSWAP) {
    rp->p_flag =& ~SSWAP;
    aretu(u.u_ssav);
}
/*
* The value returned here has many subtle implications.
* See the newproc comments.
*/
return(1);
```

The general usefulness of evolutionary activities and the productivity of knowledge in highly divergent taxa with included DNA amplification protocol.

IRENE MOON

The concept of Monophyly or the concept of organism relatedness, when speaking on an evolutionary level, has discordant meaning based on the level of the taxa involved. A common ancestor and all of its descendants is a murky term when examining extant taxa. The number of extinctions and the frequency of convergent evolution make the monphylic hypothesis based on presently living organisms tentative. It is well known, for example, that the horse came about in closer to present evolutionary time than the six-legged arthropods. But this does not confirm that the horse had in any way evolved from said insectoid. Our present arthropods (Insecta) range from the incredibly primitive, to the highly evolved; the specialized arthropods may even evolve in just a few of our comparatively long life spans.

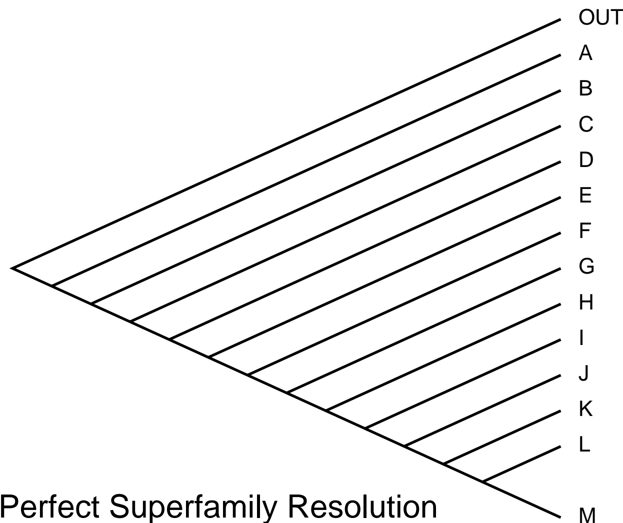
The quickly evolving Braconidae, which were thought to have undergone extensive radiation just after the boom of flowering plants and the subsequent boom of Lepidopteron pests to these plants (Cretaceous Period, 144-65 million years Ago), are often very similar in morphological structure. The species concept is quickly called into question when the visible characteristics of an organism are physically similar to its sister species, and the ability for the organism to reproduce with its sister organism is only blocked by a mountain range or similar geographic boundary.

Would one only suspect that the idea of a species remains simply a continuum and non-cynical question of belief? Typically the most popular litmus test for a species is if there is low gene flow between a taxon and the other species in question. Organisms are allowed to be considered separate species even if low gene flow exists. This takes into account may of the bizarre lifestyles of the hybrids, or those that are the reproductive offspring of two separate species, but are not reproductive themselves. The continuum for the amount of gene flow, although not exact in principle, also accounts for "species in the making." It is well known that for multiple reasons a common housefly from its far northern range cannot reproduce successfully with a member of the same species from its far southern range. However, genetic information is passed down through the entirety of its range. In this case the Musca domestica from up north is just considered behaviorally modified from its southern population. Most likely this modification can be attributed to the weather. The interest in the common house fly is not generally of great public interest, except in the creation of improved swatters. One could argue, that if M. domestica was a lovely butterfly, its northern and southern populations would at least be granted the dignity of subspecies classification.

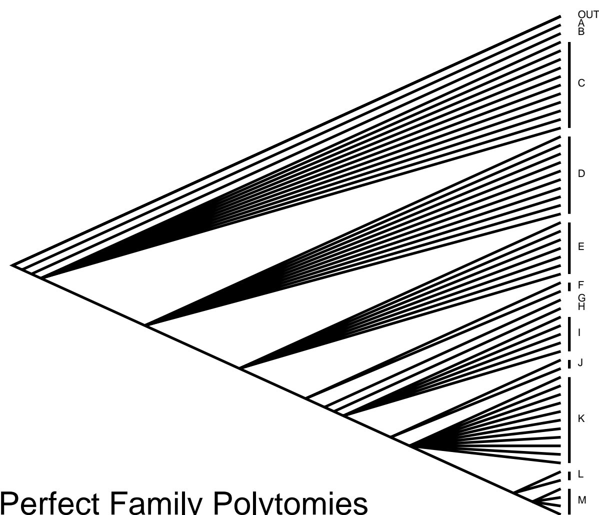
Similarity of morphological structure and the lack of adequate numbers of ancestors in the fossil record lead to a variety of difficult questions;

1. At which point is a species considered a species?
2. During the congestion of diversification, can we really rely on the principles of Parsimony?
3. If we can be clear that the most parsimonious evolutionary route prevails, do we dare create a hypothetical ancestor to resolve the close morphological relationships among the taxa in the diversifying clade?

The basal symphytan, with their many celled wing are often recorded from the fossil record. They are obviously in a different taxanomic group that the modern chalcid, wing-cell less wasp. One may see the fossil record many large, hearty hymenopterans preserved in amber. The cell formation is viewed effectively by submerging the amber in generous amounts of honey or molasses and then holding them in place beneath a coverslip, mounted atop a piece of wax. The amber is best lit from above and below with diffused light. Honey, molasses and maple syrup are all adequate for viewing as these substances change the way the light refracts, allowing clear viewing of the amber’s contents. Natural molasses, being a bit more opaque is not preferred. Although the Symphyta are basal to the chalcid, using a symphytan as an outgroup for an analysis of the Chalcidoidea is ineffective. The outgroup is used for comparative purposes. By comparing a family to its nearest relative we can determine which characters present are ancestral for that family, potentially polarizing the cladogram. The effectiveness of this comparison is proportionally informative to the relatedness of the outgroup comparison. For example, in the most recent analysis of the Cynipoidea, Diapriidae was used as the outgroup, based on unique features they both exhibit. The M+Cu vein of the hind wing is concave and the basal flagellomere of the male antenna contain pores that are used during courtship. These characters suggest a sister group relationship between them because the characters are absent in the Parasitica. The establishment of a basal group allows for the hypothesis involving which organism was derived from which, based on what trait, the esoteric goal of Systematics. Without analysis using outgroup comparison, the threat is the perfect polytomy, and zero resolution in your tree.



Perfect Superfamily Resolution



Perfect Family Polytomies

Resolution to subfamily (Ichneumonoidea), in relation to the rest of the Parasitica, does not guarantee resolution on the Braconidae family level. This requires correct identification of family relationships inside the entire order of Hymenoptera, allowing for correct outgroup comparison. However, although often dismissed as biased research, the hypothetical ancestor could act as polarizing influence in analysis where the sister group is tentative.

Even if security in our natural outgroups has been established, the highly diversified Ichneumonoidea remain problematic due to continuous morphological characters, and poorly differentiated character systems. Fortunately, techniques in the field of Systematics allows for comparison on a molecular level. The AGCT base paring of a nucleotide often resolves relationships where genitalia dissections and other reproduction specific morphological analysis fail. For highly diversified groups, such as within the Braconidae, Ribosomal DNA proves most successful. CO1 is found only to be useful at the level of Superfamily, due to its highly conserved nature. EF1alpha, which has multiple variable regions on the genome that can yield information for recently diversified species; however, generally the gene is difficult to work with. Below is one of the best general overviews of DNA amplification and general advice for laboratory etiquette. Such techniques are already standard within systematics labs and provide an important tool to solve many of the difficult problems of which morphological outgroup comparisons have failed to resolve.

OVERVIEW OF STEPS IN DNA SEQUENCING

1. Extract genomic DNA
2. Amplify known gene region
3. Verify successful PCR amplification
4. Clean PCR products
5. Quantify DNA concentration
6. Cycle-sequence
7. Precipitate cycle-sequenced products and submit them

Protocol for PCR using Taq DNA Polymerase from Fermentas Life Sciences protocols.

PCR allows the production of more than 10 million copies of a target DNA sequence from only a few molecules. The sensitivity of this technique means that the sample should not be contaminated with any other DNA or previously amplified products (amplicons) that may reside in the laboratory environment. Some of the most commonly amplified extras are yeasts, human (from dead flake skin) and E. coli (from poor hygiene). Best to avoid these embarrassments.

Guidance in Avoiding Contamination

- DNA sample preparation, reaction mixture assemblage and the PCR process, in addition to the subsequent reaction product analysis, should be performed in separate areas.
- A Laminar Flow Cabinet equipped with a UV lamp is recommended for preparing the reaction mixture.
- Fresh gloves should be worn for each PCR step.
- The use of dedicated vessels and positive displacement pipettes or tips with aerosol filters for both DNA sample and reaction mixture preparation, is strongly recommended.
- The reagents for PCR should be prepared separately and used solely for this purpose. Autoclaving of all solutions, except dNTPs, primers and Taq DNA Polymerase is recommended. Solutions should be aliquoted in small portions and stored in designated PCR areas. Aliquots should be stored separately from other DNA samples.
- A control reaction, omitting template DNA, should always be performed, to confirm the absence of contamination.

Contamination often goes unnoticed

These are only rough guidelines. Detailed instructions about PCR laboratory setup and maintenance may be found in PCR Methods and Applications, 3, 2, S1-S14, 1993.

Preparation of Reaction Mixture

To perform several parallel reactions, we recommend the preparation of a master mix containing water, buffer, dNTPs, primers and Taq DNA Polymerase in a single tube, which can then be aliquoted into individual tubes. MgCl2 and template DNA solutions are then added. This method of setting reactions minimizes the possibility of pipetting errors and saves time by reducing the number of reagent transfers.

Reaction Mixture Set Up

- Gently vortex and briefly centrifuge all solutions after thawing.
- Add, in a thin-walled PCR tube, on ice:

Reagent	Final Concentration	Quantity for 50µl of reaction mixture
Sterile deionized water	-	variable
10X PCR buffer	1X	5µl
2mM dNTP mix	0.2mM of each	5µl
Primer I	0.1-1µM	variable
Primer II	0.1-1µM	variable
Taq DNA Polymerase	1.25u/50µl	variable
25mM $MgCl_2$	1-4mM	variable*
Template DNA	10pg-1µg	variable

Table for selection of 25mM MgCl2 solution volume:

Final concentration of $MgCl_2$ in 50µl reaction mix, mM	1.00	1.25	1.50	1.75	2.00	2.50	3.00	4.00
Volume of 25mM $MgCl_2$, µl	2.0	2.5	3.0	3.5	2.0	3.0	6.0	8.0

- Gently vortex the sample and briefly centrifuge to collect all drops from walls of tube.
- Overlay the sample with half volume of mineral oil or add an appropriate amount of wax. This step may be omitted if the thermal cycler is equipped with a heated lid.
- Place samples in a thermocycler and start PCR.

Composition of the Reaction Mixture

1. Template DNA.

Usually the template DNA amount is in the range of 0.01-1ng for plasmid or phage DNA and 0.1-1µg for genomic DNA, for a total reaction mixture of 50µl. Higher template DNA amounts usually increase the yield of nonspecific PCR products, but if the fidelity of synthesis is crucial, maximal allowable template DNA quantities in conjunction with limiting number of PCR cycles should be used to increase the percentage of "correct" PCR products. Nearly all routine methods are suitable for template DNA purification. Although even trace amounts of agents used in DNA purification procedures (phenol, EDTA, Proteinase K, etc.) strongly inhibit Taq DNA Polymerase, ethanol precipitation of DNA and repetitive treatments of DNA pellets with 70% ethanol is usually effective in removing traces of contaminants from the DNA sample.

2. Primers.

Guidelines for primer selection:

- PCR primers are usually 15-30 nucleotides in length. Longer primers provide sufficient specificity.
- The GC content should be 40-60%. The C and G nucleotides should be distributed uniformly within the full length of the primer. More than three G or C nucleotides at the 3'-end of the primer should be avoided, as nonspecific priming may occur.
- The primer should not be self-complementary or complementary to any other primer in the reaction mixture, in order to avoid primer-dimer and hairpin formation.

—The melting temperature of flanking primers should not differ by more than 5℃, so the GC content and length must be chosen accordingly.

—All possible sites of complementarity between primers and the template DNA should be noted.

—If primers are degenerate, at least 3 conservative nucleotides must be located at the primer's 3'-end.

—Estimation of the melting and annealing temperatures of primer: If the primer is shorter than 25 nucleotides, the approx. melting temperature (Tm) is calculated using the following formula:

$$Tm = 4 (G + C) + 2 (A + T)$$

G, C, A, T - number of respective nucleotides in the primer. Annealing temperature should be approx. 5°C lower than the melting temperature.

If the primer is longer than 25 nucleotides, the melting temperature should be calculated using specialized computer programs where the interactions of adjacent bases, the influence of salt concentration, etc. are evaluated.

3. MgCl2 concentration.

Since Mg2+ ions form complexes with dNTPs, primers and DNA templates, the optimal concentration of MgCl2 has to be selected for each experiment. Too few Mg2+ ions result in a low yield of PCR product, and too many increase the yield of non-specific products and promote misincorporation. Lower Mg2+ concentrations are desirable when fidelity of DNA synthesis is critical. The recommended range of MgCl2 concentration is 1-4mM, under the standard reaction conditions specified. In our experiments, at a final dNTP concentration of 0.2mM, a MgCl2 concentration ranges of 1.5±0.25mM (in traditional PCR buffer) and of 2.0±0.5mM (in PCR buffer with (NH4)2SO4) are suitable in most cases. If the DNA samples contain EDTA or other chelators, the MgCl2 concentration in the reaction mixture should be raised proportionally.

4. dNTPs.

—The concentration of each dNTP in the reaction mixture is usually 200μM. It is very important to have equal concentrations of each dNTP (dATP, dCTP, dGTP, dTTP), as inaccuracy in the concentration of even single dNTP dramatically increases the misincorporation level.

—When maximum fidelity of the PCR process is crucial, the final dNTP concentration should be 10-50μM, since the fidelity of DNA synthesis is maximal in this concentration range. In addition, the concentration of MgCl2 should be selected empirically, starting from 1mM and increasing in 0.1mM steps, until a sufficient yield of PCR product is obtained.

5. Taq DNA Polymerase.

Usually 1-1.5u of Taq DNA Polymerase are used in 50μl of reaction mix. Higher Taq DNA Polymerase concentrations may cause synthesis of nonspecific products. However, if inhibitors are present in the reaction mix (e.g., if the template DNA used is not highly purified), higher amounts of Taq DNA Polymerase (2-3u) are helpful in obtaining a better yield of amplification products.

6. Reaction overlay.

If necessary, the reaction mixture can be overlaid with mineral oil or paraffin (melting temperature 50-60°C) of special PCR grade. One-half of the total reaction volume is usually sufficient.

Temperature Cycling

Amplification parameters depend greatly on the template, primers and amplification apparatus used.

1. Initial Denaturation Step.

The complete denaturation of the DNA template at the start of the PCR reaction is of key importance. Incomplete denaturation of DNA results in the inefficient utilization of template in the first amplification cycle and in a poor yield of PCR product. The initial denaturation should be performed over an interval of 1-3min at 95°C if the GC content is 50% or less. This interval should be extended up to 10min for GC-rich templates.

If the initial denaturation is no longer than 3min at 95°C, Taq DNA Polymerase can be added into the initial reaction mixture. If longer initial denaturation or a higher temperature is necessary, Taq DNA Polymerase should be added only after the initial denaturation, as the stability of the enzyme dramatically decreases at temperatures over 95°C.

2. Denaturation Step.

Usually 0.5-2min denaturation at 94-95°C is sufficient, since the PCR product synthesized in the first amplification cycle is significantly shorter than the template DNA and is completely denatured under these conditions. If the amplified DNA has a very high GC content, denaturation time may be increased up to 3-4min. Alternatively, additives facilitating DNA denaturation - glycerol (up to 10-15 vol.%), DMSO (up to 10%) or formamide (up to 5%) - should be used. In the presence of such additives, the annealing temperature should be adjusted experimentally, since the melting temperature of the primer-template DNA duplex decreases significantly. If additives are used, the amount of Taq DNA Polymerase in the reaction mix should be increased, because DMSO and formamide, at the suggested concentrations, inhibit the enzyme approx. 50%. Alternatively, a common way to decrease the melting temperature of the PCR product is to substitute dGTP with 7-deaza-dGTP in the reaction mix.

3. Primer Annealing Step.

Usually the optimal annealing temperature is 5°C lower than the melting temperature of primer-template DNA duplex. Incubation for 0.5-2min is usually sufficient. However, if nonspecific PCR products are obtained in addition to the expected product, the annealing temperature should be optimized by increasing it stepwise by 1-2°C.

4. Extending Step.

Usually the extending step is performed at 70-75°C. The rate of DNA synthesis by Taq DNA Polymerase is highest at this temperature (2-4 kb/min), and a 1min extending time is sufficient for the synthesis of PCR fragments up to 2 kb. When larger DNA fragments are amplified, the extending time is usually increased by 1min for each 1000 bp.

5. Number of Cycles.

The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. For less than 10 copies of template DNA, 40 cycles should be performed. If the initial quantity of template DNA is higher, 25-35 cycles are usually sufficient.

6. Final Extending Step.

After the last cycle, the samples are usually incubated at 72°C for 5-15min to fill-in the protruding ends of newly synthesized PCR products. Also, during this step, the terminal transferase activity of Taq DNA Polymerase adds extra A nucleotides to the 3'-ends of PCR products. Therefore, if PCR fragments are to be cloned into T/A vectors, this step can be extended to up to 30min.

Morals in Roadside Picnic - Dave Fischer

Roadside Picnic was written by Arkady and Boris Strugatsky in 1977. Largely unread outside the Eastern Bloc, it is never the less famous for being the basis for Tarkovsky's film "Stalker". Although revolving around an extraterrestrial visit to earth, this book is serious literature, and is in no way comperable to western science fiction.

The plot of the story very briefly is that aliens visited earth a few decades ago, and left without making contact. The spots where they landed are now called the "Zones", and are guarded by the UN. Entering the Zone is extremely dangerous - aside from random alien artifacts that can kill, the very laws of nature seem to have been disrupted locally. Nothing is as you would expect, and every step is risking your life.

Since any item taken from the Zone is under strict UN control, there is a huge black market for artifacts smuggled out by the so-called "Stalkers". The Stalkers have short lifespans, mutant children, and a weakened relationship with sanity. The price they pay for their black market income is very very high.

"Roadside Picnic" follows the life of one such Stalker, "Red" (Redrick Schuhart) as he steals from the Zone, spends time in jail, tries to work ligitimately for the UN research facility, and gradually gets pulled into more and more dangerous and destructive criminal activities. Even as he becomes indirectly responsable for dozens of deaths, and directly for one cold-blooded murder, he remains a sympathetic character.

It is a complex story, and a compelling story, because although it deals with bizarre situations (incomprehensable alien artifacts) it is extremely realistic. The world is complex, not just an flimsy excuse for the author's plot devices. The characters are complex and convincing - there is no simple good/evil here - everyone changes, everyone lies to themselves, and everyone bends under the pressure of their environment.

There are many facets to this book, but there are two that I find particularly interesting. One involves the main character gradually being pulled into actions that he would have absolutely condemned at the beginning. It is a subtle and chilling plot twist for the black market dealers in stolen alien technology.

The other is the metaphorical message of the entire story - living under the insane conditions of dealing with alien artifacts in the Zone represents living under the insane conditions of the Soviet state in the 1970s.

1. The Golden Ball's Price

Out of the many strange artifacts left in the Zone by the aliens, the Golden Ball is portrayed as most likely a Stalker myth through most of the book, only to turn out to be real, as the last chapter is spent reaching it. The Golden Ball grants wishes. Everyone knows the stories about it, and its existance is considered unlikely. The one man who knows where it is, and has been visiting it repeatedly over the years to recieve multiple wishes, is a Stalker named Burbridge.

Burbridge's amazing retrievals of rare artifacts from within the Zone, his inexplicable longevity, and his perfectly non-mutant children are all difficult to explain without some sort of divine intervention - wishes granted by a mysterious artifact. The horrible twist to this story however, is that the path to the Golden Ball is long and dangerous, and ends in an insurmountable obstacle - the "meatgrinder" - which grabs people, twists them into a bloody pretzel, then temporarily disappears. The only way Burbridge can reach the Golden Ball is by bringing a sacrificial victim to "feed" the meatgrinder each time he goes. Every wish he recieves costs one innocent human life.

Eventually the other Stalkers notice the pattern of assistant deaths, and threaten him:

> *Everybody was getting fed up with his tricks, and the guys had told him: you better not come back if you come back alone. That was when they began calling him Buzzard, before they used to call him Winner.*

Then Burbridge's career plans are destroyed when he is crippled by falling into the Zone's "Witchs' Jelly". He enlists Red to make one last trip to the Golden Ball, to try to bring it out. Burbridge explains the only way to get past the last obstacle:

> *"This is one place, Red, that you can't go to alone. Like it or not, you'll have to take somebody with you. I can give you one of my people who's expendable."*

Burbridge has revealed his secret to another, and convinced him to go along, even though it means becoming a murderer. Unknown to Burbridge, Red winds up with Burbridge’s son Arthur as his victim/assistant. Perhaps as revenge for being manipulated into becoming a murderer, Red finds a way for Burbridge to be victimized by the situation as well.

The whole way in, Red must work with Arthur, encouraging him and helping him so they can survive the first part of the journey. As they near the end, Red’s attitude changes, and he tries to distance himself from the kid he must trick into a violent death. He stops viewing him as a person, and sees him as a "thing".

> *Redrick was not listening. What that thing was saying no longer had any meaning. It had no meaning before, either, but before it was a person at least. And now, it was like a talking key, a key to open the way to the Golden Ball. Let it talk.*

After watching the meatgrinder slowly kill Arthur, and in a daze from the horrors of the day’s journey - the horrors of the Zone, but more importantly, the horror of his own behaviour - Red approaches the Golden Ball. As he walks past the spot where the meatgrinder resides, he looks at the remains of Burbridge’s assistants, scattered across the ground:

> *Maybe each splotch represented a person, or one of Buzzard’s wishes. That one there was Buzzard coming back alive and unharmed from the basement of Complex #7. That bigger one over there was Buzzard getting the wriggling magnet out of the Zone unscathed. And that icicle was the luxurious Dina Burbridge, who resembled neither her mother nor her father. And that spot there was Arthur Burbridge, unlike his father and mother, Artie, the handsome son, their pride and joy.*

Ironicly, Burbridge had killed a man in order to get a healthy son, and his son is sacrificed by Red in the exact same way.

2. Life in the USSR

The moral of this story is that man can adapt to living under completely irrational conditions, but it takes a terrible toll. The conditions literally described in the story are the alien artifacts left behind in the "Zone", but this is a metaphor for the conditions of day-to-day life under Communist rule in the Soviet Union.

The insanity of the system forces good, normal people into a life of crime. This is accepted, and people are willing to overlook much of the behaviour their neighbors are forced into.

For example, Red's friend Richard Noonan knows that Red is responsable for getting the "Witchs' Jelly" to the researchers, resulting in thirty-five deaths and over a hundred injuries, but ignores it. He knows conditions outside of Red's control forced him into the situation, and he knows that he himself would do equally immoral acts if he found himself in the wrong place at the wrong time. They discuss the disaster, both knowing full well that it was Red's fault, and both feigning ignorance.

> *Noonan understood why Redrick was bringing up the topic. He threw up his hands in dismay.*
>
> *"Are you kidding? Did you know what happened with that jelly? Have you heard of the Curligan Labs? There's this little private supplier... So they got themselves some jelly..."*
>
> *He told him about the catastrophe. And about the shocking fact that they never tied up the loose ends, never found out where the lab had gotten it. Redrick listened, feigning distraction, clucking his tongue, and shaking his head. He decisively splashed more whiskey into their glasses.*

It is the condition of living under a completely irrational system that is more important though. Later in the same chapter, Noonan is sitting at a table with Red, with Red's mutant daughter "Monkey", and Red's long-dead father, resurrected as a friendly zombie ("moulage") by alien forces in the Zone:

> *Noonan started in on institute business, and while he was talking, Monkey appeared noiselessly at the table by the old man. She stood there with her hairy paws on the table and then in a perfectly childlike way, she leaned against the moulage and put her head on his shoulder. Noonan went on chatting but thought, as he looked at those two horrors born of the Zone: My God, what else? What else has to be done to us before we understand? Isn't this enough? But he knew that it wasn't. He knew that millions upon millions of people knew nothing and wanted to know nothing, and even if they found out would ooh and aah for five minutes and then go back to their own routines. It was time to go, he thought wildly. The hell with Burbridge, the hell with Lemchen, and the hell with this goddamned family!*

This seems to be refering to the fact that if everyone living under a brutal and insane government decided that enough was enough, they could put an end to it. But everyone instead tries to make the best of it, and pretend that things are reasonable. Thus everyone helps perpetuate the system that they all hate. Or it's a reference to people in other countries ignoring their plight. Perhaps it is meant to be taken either way.

And finally - Red's request when he finally approaches the Golden Ball which grants wishes:

> *HAPPINESS FOR EVERYBODY, FREE, AND NO ONE WILL GO AWAY UNSATISFIED!*

This is the seemingly futile dream of men living under tyranny. Freedom and happiness. Their optimism long since having been completely crushed, the only way they could imagine it being fulfilled is from some sort of genie in a bottle that magically makes wishes come true.

PROVIDENCE MACHINES
#4 - Warming 2004

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Irene Moon is a researcher in insect systematics and founder of the Begonia Society; whose primary concern is your personal development and the promotion of laboratory aesthetics.

"Greg Brotherton is a sculptor who recycles retro-futuristic vaccuum cleaners and other industrial detritus into breathtakingly cool, highly polished rayguns and robots." - Cory Doctorow

The "you are not expected to understand this" code fragment is a legendary comment from a bit of early Unix kernel code, written by developers at Bell Labs in the mid 70s.