Using Genetics to Breed Champion Racing Pigeons

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The Rule of Seven

- There are seven factors which determine how well you and your birds will perform in pigeon racing.
- **One is beyond your control**.
- **Five are so well perfected within the sport that they have become essentially pass/fail.** You either cover them competently or you are virtually eliminated from the winning positions even before the race starts.
- **One has almost no limit** to its potential and is **largely unrealized** by most fanciers.

The Rule of Seven

1) Beyond our control – **Luck**. Good luck, bad luck, hawks, wires, wind direction, basket position on the truck, bad weather along the course, good weather along the course when we entered a tough weather bird, and on and on and on. It affects us all and so we should just get over it and move on to what we can control.
2) **Condition** (pass/fail)
3) **Training** (pass/fail)
4) **Fuel (aka Nutrition)** (pass/fail)
5) **Motivation** (pass/fail)

The Rule of Seven

6) **Health** (pass/fail though too many flyers are still failing on this one)

7) **Genetics**

When Louis Van Loon was asked “What methods do you use to get those kind of results?” he looked sternly at the gentleman and said, “Remember this, there is only one thing that is important – good pigeons, nothing else.”

Moving On to Factor Seven (Genetics)

- While environmental factors will always play a major part in contributing to race performance, the **genetic component is both real and significant**.
- Genetic progress **takes time, focus and effort**, but it is completely possible.
- Breeders who seriously commit to genetic improvement now, have the potential to enjoy a sizable competitive advantage for years to come.

Racing Ability is Polygenic

- Many fanciers are familiar with the Punnett Square for visualizing the possible outcomes of a particular mating.
- Here is a Punnett Square for a mating of a Blue Bar hen and a heterogygous Blue Check cock (where C is the allele for Check and + is the allele for Bar).
- On average 2 out of every 4 offspring will be Blue Bars and 2 will be Blue Checks.

Sire's sperm Dam's egg	C	+
+	+ C	+ +
+	+ C	+ +

Racing Ability is Polygenic

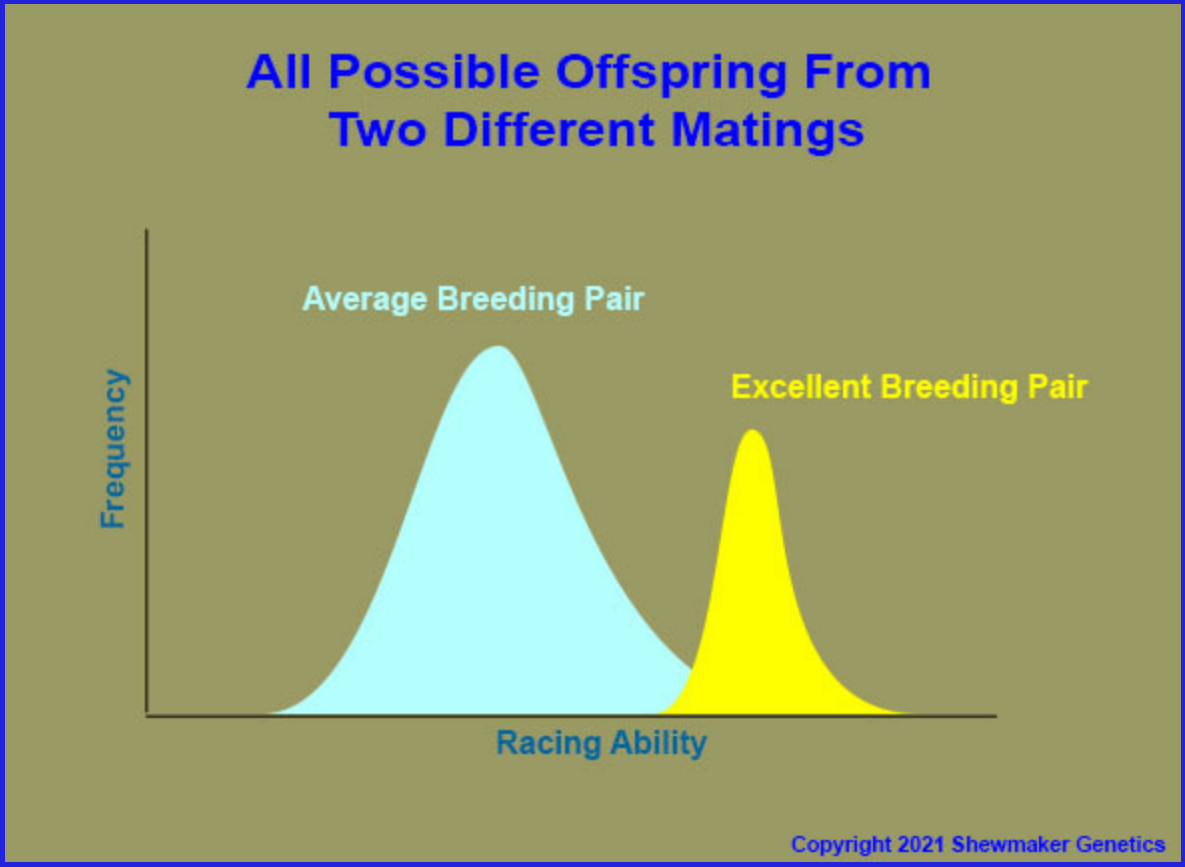
- However, there are many genes that influence racing performance (probably at least 100).
- The visual representation of all possible offspring of a mating for a polygenic trait is a bell curve:

Racing Ability is Polygenic

- Even though two siblings have the same parents, there **can** be a significant difference in their racing ability,
- AND there **can** be a significant difference in their genetic ability to pass racing ability on to the next generation.

Racing Ability is Polygenic

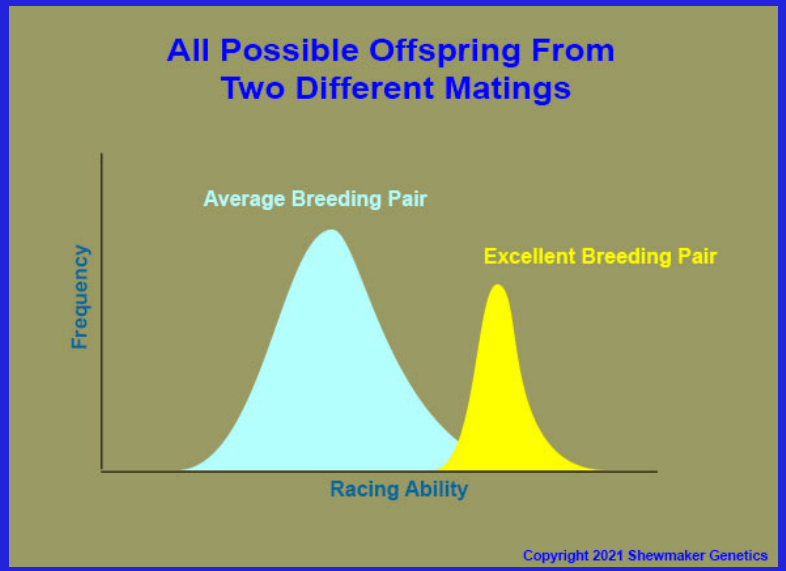
Just as each individual pigeon has a unique genetic makeup, each mating has a unique bell curve of possible offspring.



Racing Ability is Polygenic

These different bell curves vary in two important ways:

- The **center** point of the curve gives us an indication of the average racing ability for that particular mating
- The **width** of the curve is an indication of the uniformity of the offspring produced:
    - A mating with a wide curve produces a **diverse** set of offspring that have great variation in their racing ability.
    - A mating with a narrow curve produces offspring that are more **similar** in their racing ability.



Genes Affecting Racing Ability

While we can not currently identify all of the specific 100 or so genes that influence racing performance, we can group them into several functional traits that we can relate to race performance.

These traits are shown on the next slide.

Notice that seven of the eight can not be evaluated by “grading” or handling of the pigeon. Only Number 2 is related (at least partially) to physical qualities that might be successfully evaluated by “grading”. These would include feather quality, wing structure, muscle structure and so on.

As much as we might think that perfect conformation is all that is needed for a champion racer, the reality is that (while conformation certainly helps), it is but a small portion of all that is important.

Genes Affecting Racing Ability

There are several distinct traits which positively influence racing performance. All of these traits are strongly influenced by the environment, but they also have a significant genetic component. Winning birds have the:

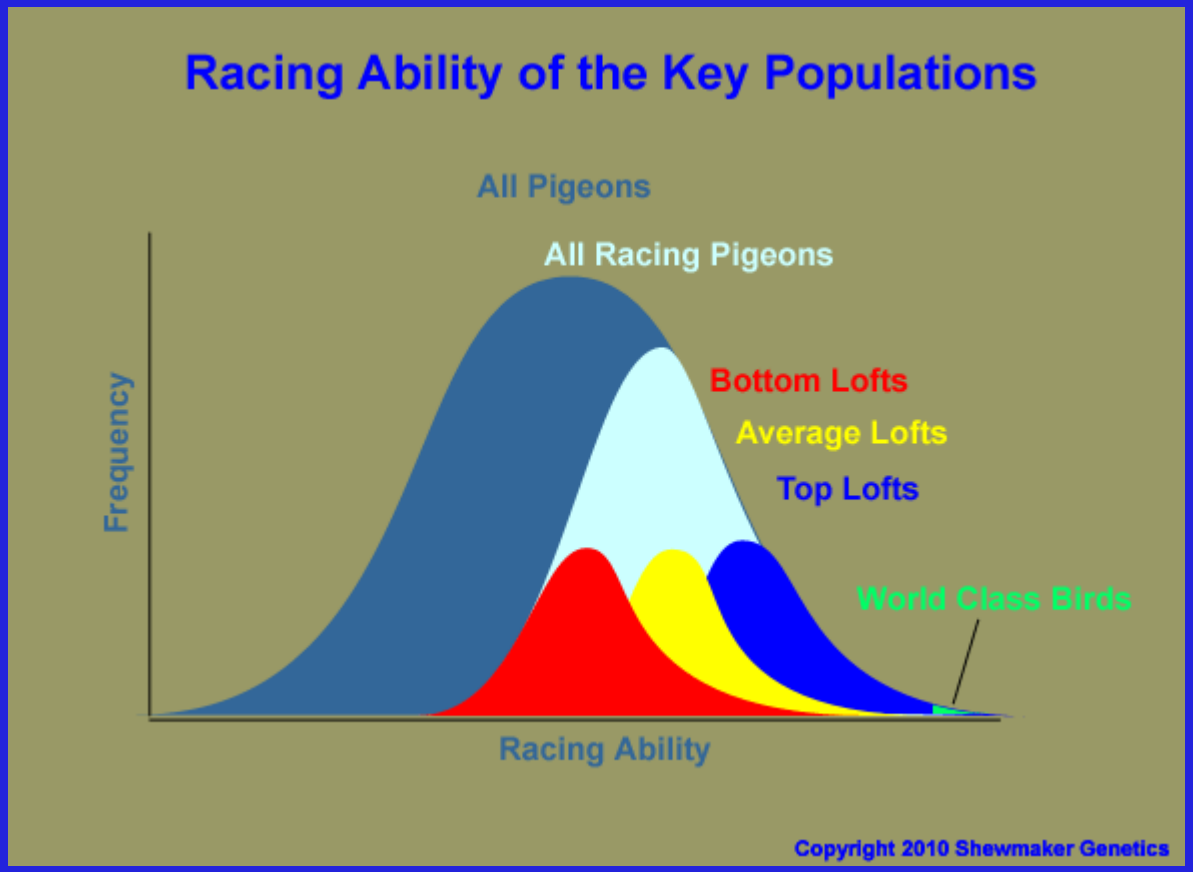
1) **ability to orient** quickly at the time of release AND maintain the proper orientation on the flight home.
2) **ability to fly** at a speed and for a duration that is competitive with the rest of the birds in the race. Many sprint birds, for example, just do not have the tools for competing in a long distance race.
3) **desire to want to get home quickly** (as opposed to just plodding along until they get there).
4) **intelligence to resolve challenges** that inevitably arise at some point during at least some races (*i.e.* strong winds or a storm that breaks up the flock and blows them off course).
5) **ability to learn** from their experiences and their mistakes.
6) **mindset of a leader** as opposed to that of a follower (which is somewhat at odds with their normal gregarious nature).
7) **willingness to take risks** such as starting for home before the pack is ready or to break from a group during the race.
8) **strongest possible homing instinct** so that they return home even on races where they don't place (birds that come home after disasters are able to race another day!)

Manage the Gene Pool

- Understanding genetics can be challenging.
- Many people get lost in the minutia of the details - DNA, genes, alleles, cross over, mutations and the like.
- While such details are indeed import, it is **much more important** to understand the big picture. Remember, it is fine to understand how a watch works, but most people are better served by simply knowing how to tell time.
- In general, don't think in terms of individual genes, individual chromosomes or even individual birds. Everything should be approached from the point of view of the *population* of racing pigeons – specifically those in your loft.
- Focus on managing the **gene pool** in your loft.

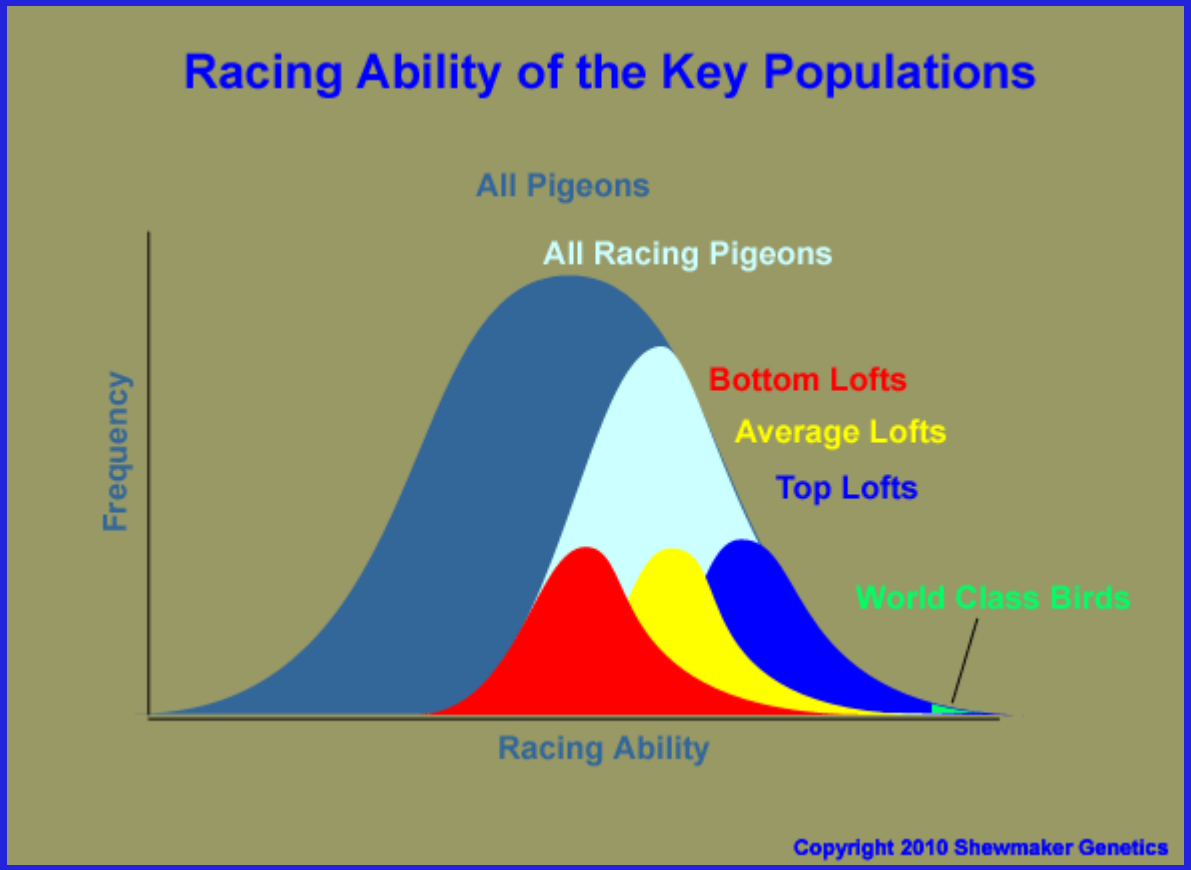
Manage the Gene Pool

- The bell curve can also be used to represent the racing ability of a gene pool.



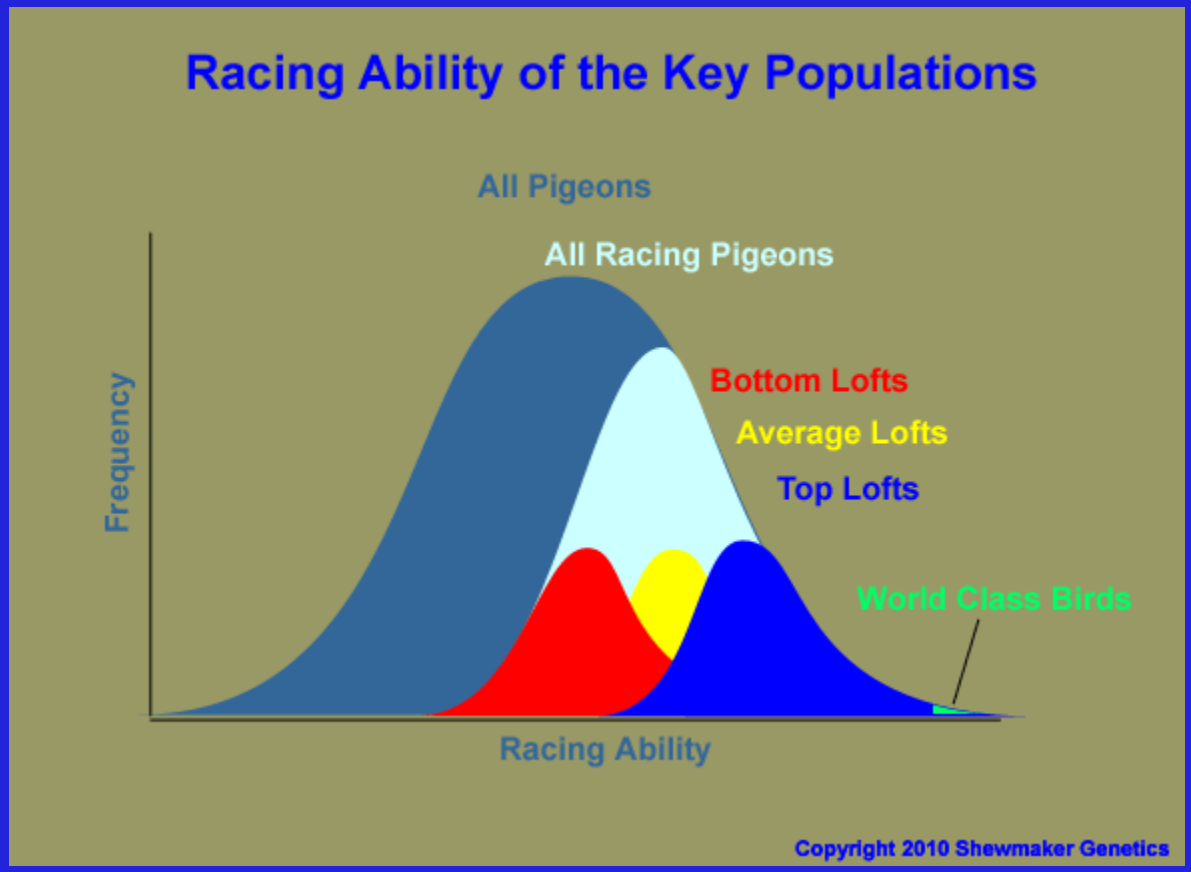
- The next five slides are the most important of the whole seminar.

Manage the Gene Pool



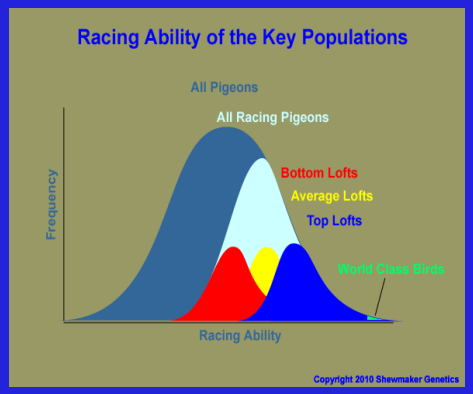
- Notice that the “Bottom Lofts” and most of the “Average Lofts” may not even have the necessary genes in their pool to breed world class birds.

Manage the Gene Pool



- But also notice that in the “Top Lofts”, few of the birds are “World Class” and many are on a par with the “Low” and “Average” lofts.

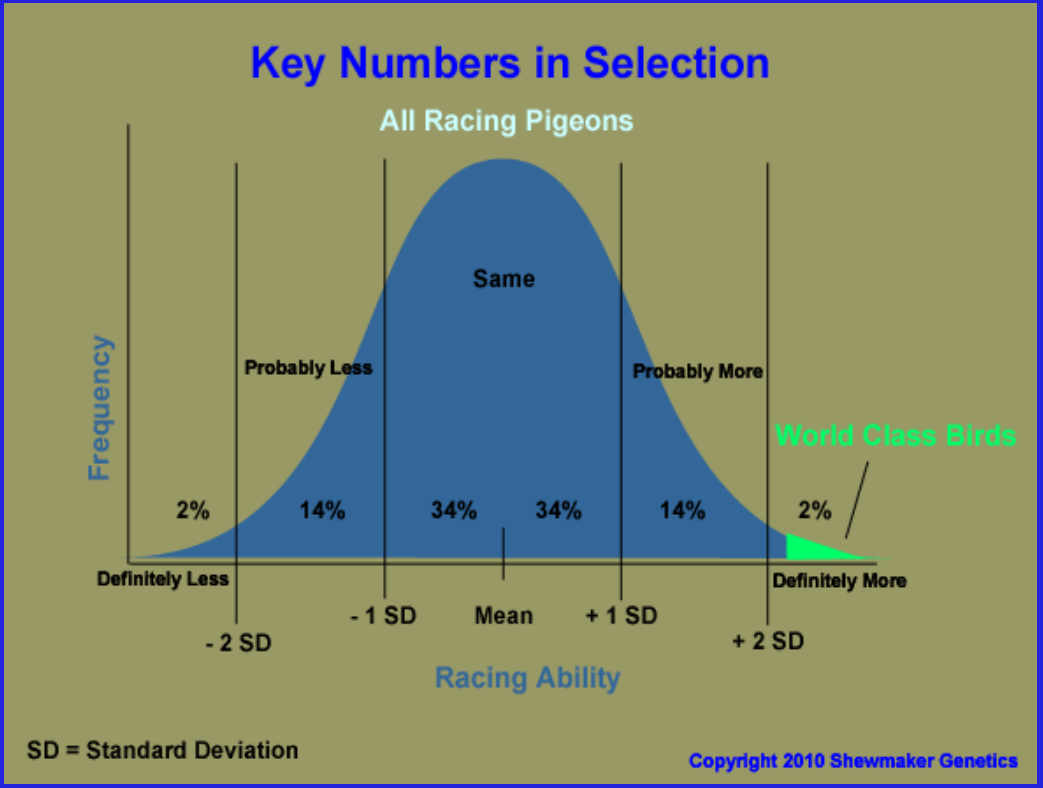
Manage the Gene Pool



- **Here is the hard cold fact** – most of our pigeons are not genetically up to our assumptions and expectations.
- ***IF*** you have a "Top Loft" complete with a few "World Class" pigeons, you ***MIGHT*** produce **1 in 10** birds which should be kept to breed the next generation.
- If you are in the "Average Loft" category truly outstanding birds are probably closer to 1 in 100 and in the "Bottom Lofts" it is closer to 1 in 1000 or maybe even 1 in 10,000.

Manage the Gene Pool

- If you aren't selecting your breeders from at least the top 16% *you probably aren't selecting at all*.



- **If you really want to make progress you need to be selecting at least from the top 2%.**

Manage the Gene Pool

- **UNDERSTAND THIS**: birds selected from the top 2%, breed offspring who have their own bell curve. While the mid point of this new bell curve will very likely be shifted right, it will NOT consist only of birds that are as good as the 2% ones you selected. The new bell curve will likely extend well into (and perhaps beyond) the mediocre range.
- If the 2% birds you selected to breed are indeed very good breeders, they still are, at best, going to produce only 1 in 10 that will be good enough to carry your breeding effort forward.
- So, an untested breeder “Bred for Stock” has at least a 90% chance of being a mistake. Stock untested youngsters from a pair of “bred for stock” breeders and the odds the wrong ones were selected jumps to at least 99%.
- There is a place for “Bred for Stock” but the practice has its risks and should be carefully managed (like following up with testing of the offspring produced to verify you stocked the right one).

The Progress Equation

- Your ability to make genetic progress and the speed at which you make this progress is **absolutely** related to these four factors (*memorize this slide*!):
  - The **accuracy** of your selection
  - The **intensity** of your selection
  - The **time** interval over which you do the selection
  - The **frequency** of the desired alleles in your gene pool

The Progress Equation

- The **accuracy** of your selection
  - *So if you think toe color is related to superior racing and this is what you select for, you are probably not really doing any selection at all with respect to racing ability. In my view the most accurate selection criteria (by a wide wide margin) is **race results to the same loft**. Nothing else comes close.*
- The **intensity** of your selection
  - *Selecting from the top 16% is far less intense than selection from the top 2% which in turn is far less intense than selecting from the top 1%*
- The **time** interval over which you do the selection
  - *One season is not enough, but two or three will surprise you. Changing to a new fad (or a new family) every few years will doom any real progress*
- The **frequency** of the desired alleles in your gene pool
  - *The desired alleles must actually be in the gene pool of your loft*

The Progress Equation

- Progress = (accuracy)(intensity)(time)(frequency)
- If you want to **increase** the speed of the genetic progress in your loft, you need to increase one or more of these four factors.

  If you **decrease** one, you will have to increase one or more of the others just to maintain the rate of your current progress.

  If any of these factors equals **zero**, you will make zero progress.

  - The **accuracy** of your selection
  - The **intensity** of your selection
  - The **time** interval over which you do the
  - The **frequency** of the desired alleles in your gene pool

The Progress Equation

- Revisiting our “Bred for Stock” discussion, stocking the wrong bird means your selection accuracy was zero.
  - So while you got the wrong bird, the bell curve for your population would not likely have shifted (i.e. you were neither selecting for or against the desired alleles).
  - If you have been guilty of too much “Bred for Stock” in your breeding program, realize that the key alleles are probably still in your gene pool, just not in one bird and not in as high a frequency as we are trying to achieve.
  - To recover from such a mistake, start testing 100% of your youngsters and retain for breeding **only** the very best (the top 2% would be my recommendation).

The Progress Equation

- Revisiting our “Bred for Stock” discussion (continued) ...
  - Realize though that in a small loft, low frequency alleles can be lost from the gene pool through non-selection events such as race losses and deaths.
  - For this reason, it is critical to avoid scenarios where selection accuracy is very low or zero.
  - If you want to acquire birds from another loft where too much “Bred for Stock” has occurred, don't acquire just one or two birds. Such a sample of their gene pool may not be sufficient to capture the desired alleles. Acquiring a kit of 10 less expensive squeekers would be a much better idea than one or two higher priced “Bred for Stock” birds.

The Progress Equation – A Powerful Example

- Lets look at an example. We will greatly decrease the generation time so that we can see (quickly) how a significant **time interval** (i.e. 7 generations) can bring about dramatic progress in meeting a breeding objective.
  - My awareness of this example has given me a tremendous advantage in breeding racing pigeons. It gave me the nerve to trust the Progress Equation and to do my “60 x 60” program where I get my test birds out to 60 miles by 60 days of age.
  - As pigeon breeders we usually think of one generation as a year. This is how long it takes us to breed a youngster and raise it to the age when it can breed.
  - Bacteria under ideal laboratory conditions have a generation time of about 20 minutes!
  - This means that in a single 24 hour day, a bacterial population can progress 72 generations! This is more generations than most pigeon fanciers will be able to breed in their lifetime.

The Progress Equation – A Powerful Example

- For this example, we are going to breed some bacteria just like we breed our pigeons.
  - Just like with pigeons, we will have a breeding objective and we will select the next generation of breeders with this objective in mind. We will continue this for seven generations so that we can see just how the Progress Equation works.
  - Breeding Objective: Breed a line of bacteria that is resistant to an antibiotic.
  - First some basics about growing and breeding bacteria!

The Progress Equation – A Powerful Example

- This is what a sterile petri dish with no bacterial growth looks like. It is clear and transparent:

- This is what a petri dish looks like after spreading 300 million bacteria on it and incubating overnight. It is covered with a white coating and is opaque:

- If we only place 8 bacteria on the petri dish and incubate overnight, it will look something like this with eight white spots. Each of these spots is a colony of millions of bacteria all descended from the one bacteria initially placed at that location:

The Progress Equation – A Powerful Example

- Now lets take a solution of bacteria (generation 0) and mix it with a weak solution of an antibiotic.
- Lets then put about 300 million bacteria from this mixture on a sterile petri dish and incubate it overnight.
- The next day we will see a small number of colonies. Each of these colonies (generation 1) grew from a bacteria that possessed a mutation which made it resistant to a weak exposure to the antibiotic.

- You will notice that in this example that we are exercising extreme **selection intensity** (8 or so out of 300 million).

The Progress Equation – A Powerful Example

- Now lets take a sterile instrument and touch it to one of these generation 1 colonies and then swirl it in a sterile nutrient solution. Incubate this inoculated solution overnight.
- This time instead of a weak antibiotic solution we are going to add an antibiotic solution that is 10X stronger to the bacterial solution.
- Repeat the earlier process by placing about 300 million bacteria from this “generation 1“ solution on a sterile petri dish and incubate it overnight.
- The next day we will again see a small number of colonies. Each of these colonies (generation 2) grew from a bacteria that possessed a mutation which made it resistant to the stronger antibiotic. The mutation likely arose at some point during the previous incubation period when billions of cell divisions occurred.

The Progress Equation – A Powerful Example

- Repeating this process for 5 more generations (each cycle increasing the strength of the antibiotic by 10 fold) will result in a petri dish with a number of colonies each of which are 100% resistant to the antibiotic.
- There is a wonderful video from the the Harvard Medical School which demonstrates this example with a slightly different (but much more eloquent) setup

  www.youtube.com/watch?v=plVk4NVIUh8

- What have we learned?

The Progress Equation – A Powerful Example

- Using high levels of accuracy and intensity in our selection, it only took 7 generations to achieve a remarkable outcome.
  - The **accuracy of our selection** was pretty clear – the bacteria were exposed to the antibiotic and if they were susceptible, they died.
  - The **selection intensity** was extreme (8 or so out of 300 million).
- This example dramatically illustrates how genetic progress is indeed a function of accuracy, intensity, time and frequency.
- It also underscores the importance of selection accuracy, selection intensity and allele frequency since there isn't that much we can do to change the time interval available to each of us.

Know Your Goals and Objectives

- There are many ways to breed champion racing pigeons. No one way is necessarily better than another.
- It is more of a question as to what are your goals and objectives and what is the best way to meet them.
- The successful breeder has very specific end points in mind and a well thought out plan for getting there.
- The successful breeder does not randomly buy good birds every year and then randomly mate them, hoping to win some races.
- One of the key objectives a breeder must resolve in their mind is this - do they want to breed that **one in a million** great racing bird or do they want to breed **many** very good racing birds?

Know Your Goals and Objectives

- A **gene pool with a narrow bell curve** will tend to
  - produce a crop of offspring that are more **uniform** in their racing ability, but at the same time,
  - this **lack of variation** makes it less likely that the pool will produce those extremely exceptional racers that represent the far right of the bell curve for the population of “all racing pigeons”.
- A **gene pool with a wide bell curve** will tend to
  - produce a crop of offspring with much more variation in their racing ability (compared to the narrow pool) and
  - the good ones might be better, they will be fewer in number.

The Basic Tools

1) "Contemporary Group" data

- Only race and test data from flights to the same loft should be used for genetic evaluation. This normalizes environmental influences.
- Use relative measurements such as percentiles and not absolute measurements such as speed or race position.
- Value birds with multiple instances of notable race performances.
- Value birds whose relatives also performed well.

The Basic Tools

2) **Inbreeding and linebreeding** (a form of inbreeding)
   - Used to concentrate desirable alleles and narrow a gene pool.
   - Testing and strict culling are particularly important when inbreeding.
3) **Outcrossing**
   - Used to add specific alleles to the pool or
   - to increase diversity when progress has plateaued.
4) **Crossbreeding**
   - Used to get an edge when racing by maximizing vitality
   - Crossbred birds should not normally be put in the breeding program.

The Basic Tools

5) **DNA testing** for performance alleles

- Used to increase the frequency (ultimately to 100%) of the desirable alleles of certain genes affecting race performance (LDHA and DRD4 as of right now).
- While potentially very important, LDHA and DRD4 represent just 2 of the approximately 100 genes that influence race performance. Fanciers should be careful not to place an unreasonably heavy emphasis on these 2 genes at the expense of the other 98.
- The subject of LDHA and DRD4 testing is examined further in an appendix to this presentation.

The Basic Tools

6) Artificial Insemination

- Semen can be collected and frozen allowing us to preserve the genetics of the very elite long after their natural reproductive life is over.
- Semen can also be collected and frozen from race team cocks during and between race seasons. This is huge.
  - It allows us to preserve the genetics of the elite birds while continuing to race them and gather data. In the past we have often had to chose between stocking and racing which resulted in some birds being either
  - stocked too early before their true racing value was accurately established, or
  - sent to one too many races wherein a valuable bird was lost.

The Needed Next Step

- The sport of pigeon racing has been dramatically evolving in recent years to include many large one loft races.
- These races offer a unique opportunity for genetic improvement:
  - The birds entered into a one loft race are a good contemporary group so that the race results largely represent genetic data somewhat reasonably normalized for environmental influences.
  - If these large one loft races were to require a one generation pedigree for each bird entered into the race and
  - if an organization were to gather this data from all or many of these large one loft races,
  - it would be possible to identify the top breeding cocks and hens in the sport.
- It is long shot given the amount of work and cooperation that would be required, but it is intriguing to consider the possibilities!

A Road Map for Genetic Improvement

1. Assemble an appropriate gene pool. Don't assume though that you have to go buy new birds. While we all probably need to cull out most of what we have, the Racing Pigeon Gene Pool is very deep and until you conduct a fair test you really can't say you don't already have the right genes.
2. Roll, roll, roll the dice!!!
    - If the genes are in the pool, your job is to assemble them all in one bird.
        - Breed, test and cull until you get one. Then do it again to get another one.
        - Change the matings and do it again.
        - Use linebreeding to try to concentrate the genes of elite birds.

A Road Map for Genetic Improvement

3. For selection purposes, use only contemporary group test results (Contemporary Groups are groups of birds where the environmental factors for every member of the group are as equal as possible).
    - **Loft** results for Young Bird and Old Bird races (Combine wins are great for bragging rights and marketing, but useless for genetic selection)
    - Training tosses
    - One Loft Races
    - Your own Contemporary Group Tests

A Road Map for Genetic Improvement

4. Perform tough but fair tests. The ideal test is one where only one bird comes in first and is followed over a reasonable period of time with small drops, culminating in all (or at least most) of the birds coming home on the day.
   - The worst possible test is a smash where no one comes home.
   - The second worst test is where the vast majority of the birds come home in the first drop. (It would be an excellent result for YB or OB race or even a training toss but not for a test. A drop of 16 birds for example that score 1-16 in the club or combine speak highly of the handler, but it is really difficult to known whether you had 1 leader and 15 followers or even if you had a flock of 16 and no one bird capable of doing the same on their own.)

A Road Map for Genetic Improvement

5. Form your conclusions based on patterns, not individual results. In general, don't treat anything as significant until you have three or more noteworthy results.
   - Don't get attached to the pretty ones or the expensive ones or even the ones with a single win. If the results aren't repeatable, they probably aren't statistically significant from a genetic perspective.
   - Two noteworthy results and you may have something.
   - Three noteworthy results and you probably do.
   - Multiple noteworthy results among multiple relatives is the gold standard!

A Road Map for Genetic Improvement

6. Shoot to limit selection to top 2% or **1% if possible**

- Of course, not every bird you stock will be in the top 2%. There are many reasons for exceptions, just don't make these exceptions without well thought out and solid reasons.
- The message here is not to make it seem impossible, but to emphasize that most of the pigeons we produce and keep are not suitable for moving the flock forward, so be (much) more selective.

A Road Map for Genetic Improvement

7. When you get one of the 1% birds, know it is special and do everything you can to breed (and test) as many of its youngsters as you can. Avoid stocking without testing.
    - For 1% cocks:
        - Polygamous breeding
        - Artificial Insemination – 300 youngsters a year possible from a single cock
    - For 1% hens:
        - Foster off the eggs to pumper pairs
        - Breed to multiple cocks
    - Repeat the mating that produced the bird and variations of the mating using relatives

A Road Map for Genetic Improvement

8. Birds selected for breeding (i.e. the top 2% of racers) have only passed the first test phase. We are really looking for birds that pass Phase 3 Testing:
   - Phase 1 - Contemporary Group Testing/Competition.
   - Phase 2 – Finding birds that breed the top 2% racers
   - Phase 3 – Finding birds that breed birds that breed the top 2% racers.

A Road Map for Genetic Improvement

9. Use your tools as would a craftsman, not a hack:
   - Inbreeding & linebreeding to selectively narrow the gene pool, then
   - Outcrossing when progress has plateaued.
   - Use crossbreeding to get an edge when racing (but know this is a disadvantage for breeding).
   - Use DNA testing on your top performers to identify their genotypes for LDHA and DRD4.
   - Have a goal. Make a plan. Execute the plan. Be Observant. Keep an open mind & listen to others, but think for yourself. Be honest with yourself. Look to improve.

Closing Thoughts

- Remember genes determine the potential. Environment limits how much of that potential is realized. As people get better and better at perfecting the environmental factors (condition, training, fuel, motivation, health and luck) genetics is the one remaining **but unlimited** area in which improvement can still be made.
- This was a crash course and covered way too much information to be absorbed in one sitting. Reread these slides again in a month. They will always be available at **www.shewmaker.com**.
- I have a group on Facebook called “Shewmaker Genetics” where I occasionally post useful information about genetics and pigeon racing. It is a public group so any racing pigeon fancier is welcome to join. We have fanciers from all over the world that are members.

Appendix: DNA Testing of LDHA and DRD4

The fantastic advances in DNA technology are now available to the sport of pigeon racing!

- DNA Profiling allows us to record the genetic “fingerprint” of a pigeon. This can be very useful later for a variety of verification scenarios.
- Verification of Parentage. While a 100% verification is not possible, the use of at least 16 carefully chosen markers will allow parentage to be verified to a very reasonable degree.
- Sex determination.
- We can now determine the actual genotypes of birds for two genes that have been shown to influence race performance. More are undoubtedly coming.

Appendix: DNA Testing of LDHA and DRD4

Recent research has shown that the LDHA gene may play a very important role in racing performance of pigeons.

I believe this is a very important topic, but a strong word of caution is in order.

- First and foremost, the LDHA gene is but one of many that contribute to racing ability. Anyone who jumps off the cliff at this point and assumes that LDHA is the secret and exclusive “silver bullet” which will ensure immediate racing success, is almost certainly wrong and will likely end up being very disappointed.
- By the same token, anyone who dismisses these research results as techno babble and irrelevant to real world racing is also very likely wrong and might be missing a significant opportunity to move their gene pool dramatically forward.

Appendix: DNA Testing of LDHA and DRD4

What is it?

- LDH stands for **Lactate Dehydrogenase**, a group of enzymes that are involved in the conversion of lactate to pyruvate (and vise versa).
- LDH is found in the cells of virtually every living organism (plants, animals and even single cell organisms known as prokaryotes).
- In mammals and birds, there are three different forms of this enzyme that are largely found in specific cell types, reflecting the different functional requirements of those cells. Each type is coded for by a different gene.
- The type **A form** of LDH is found largely in muscle cells and is coded for by the **LDHA gene**

Appendix: DNA Testing of LDHA and DRD4

What is it?

- When sufficient oxygen is present, muscle cells produce energy from a metabolic process known as aerobic respiration.
- When the exercise is sufficiently intense or prolonged such that there is an **oxygen deficit**, muscle cells use an alternative anaerobic process that **produces lactate (lactic acid)**. Note that pigeons use a metabolic pathway for energy that uses fat and does not significantly produce lactate after the first hour of flight.
- For many years, it was erroneously thought that muscle fatigue during strenuous exercise was due to a build up of lactic acid. We now know that there are several factors that contribute to fatigue, but **how a cell utilizes and/or regulates lactate levels can influence race performance**.

Appendix: DNA Testing of LDHA and DRD4

What did the research find?

- In 2002, two different alleles were found in pigeons for the LDHA gene, A and B. This means the possible genotypes for LDHA in pigeons are BB, AB and AA.
- In 2006, DNA testing was used to determine the frequencies of the A and the B alleles in four groups of pigeons:
  - The group of fancy pigeons (non racing breeds) had an A allele frequency of **0.6%**.
  - A control group of race pigeons (not screened for racing results) had an A allele frequency of **6.5%**.
  - A group of race pigeons from throughout Poland (specifically screened for “top” racing results) had an A allele frequency of **20.3%**.
  - A group of race pigeons from throughout China and Taiwan (specifically screened for “top” racing results) had an A allele frequency of **21.9%**.

Appendix: DNA Testing of LDHA and DRD4

What did the research find?

- In 2014, another study was done which again demonstrated a correlation between the frequency of the A allele and race performance.
- This 2014 study also raised the possibility that the influence of the AA genotype may exceed that of the AB genotype in races under 250 miles and that the A allele may be less important in the distance races of more than 311 miles.
- At this point there are many unanswered questions. Much additional research needs to be done.

Appendix: DNA Testing of LDHA and DRD4

What does this all mean?

- In selecting for race performance, pigeon breeders have indirectly been selecting for the A allele of the LDHA gene (along with others of course that have not yet been identified). This is shown by the ten fold increase in the frequency of the A allele of the racing pigeon control group over that of the fancy pigeons in the 2006 study.
- The three fold increase in the frequency of the A allele of “elite” racing pigeons over the racing pigeon control group further supports the notion that the A allele enhances race performance.

Appendix: DNA Testing of LDHA and DRD4

What does this all mean?

- Today, the LDHA genotype of any pigeon can be determined by a DNA test. In the US, the test can be performed for $20 with the submission of a single secondary feather.
- It is now possible for the astute breeder to “fix” the A allele of the LDHA gene in their breeding flock, making its frequency 100%. They are then free to focus on additional improvement through the selection of other key genes, knowing the A allele will always be there in any birds they produce.

Appendix: DNA Testing of LDHA and DRD4

Don't forget – this is an important gene, but it is not the whole story. There are many outstanding birds (both racers and breeders) who are BB.

- Don't make the mistake of culling birds just because they do not carry the A allele.
- Think instead in terms of adding the A allele to improve existing gene pools and then increasing its frequency.

Appendix: DNA Testing of LDHA and DRD4

In 2013 I bred an incredible bird. His Contemporary Group Test record was unlike any of the thousands of birds I have tested. No other bird has had a record that was even close. He was an off the chart freak!

- His band was 3079-AU-13-SHEW and I named him “The Freak” (sorry Frank McLaughlin, I didn't know at the time you had one with the same name).
- Later when I started testing my birds for the LDHA gene, I assumed he would probably be at least AB and maybe even AA.
- Well he wasn't. He was just a BB.

Appendix: DNA Testing of LDHA and DRD4

In 2013 I bred an incredible bird... He was an off the chart freak!

- There is this natural tendency when doing gene testing to be disappointed when the results come back without the hoped for (or expected) alleles. Don't let this happen!
- There are probably a hundred (or more) genes that contribute to a pigeon's ability to race. The LDHA gene is important, but it is still just one of at least one hundred.
- If you have an outstanding family of birds and they test out as almost all BB, this is actually a very good thing. It means you have the right alleles for many of the other 99 genes. If you add the A allele for LDHA, it will be like throwing gasoline on a fire – BOOM!!!

Appendix: DNA Testing of LDHA and DRD4

In 2013 I bred an incredible bird... He was an off the chart freak!

- In 2016 I started to do some DNA testing for another gene that scientists have correlated with race performance in pigeons.
- It is the Dopamine Receptor D4 gene and is commonly called the DRD4 gene.
- It turns out “The Freak” tested CTCT for DRD4 which we will see in a few minutes is super.

Appendix: DNA Testing of LDHA and DRD4

- The DRD4 gene codes for the D4 dopamine receptor, which is a protein-coupled receptor found on the surfaces of certain cells of the central nervous system. These receptors are activated by dopamine and are part of an elaborate messaging system within the body used to regulate various neurological processes. **DRD4** stands for **D**opamine **R**eceptor **D4**.
- DRD4 has been studied in humans and various mutations of the gene have been linked to a number of neurological and psychiatric conditions such as schizophrenia, certain eating disorders, Parkinson's Disease and even some addictive behaviors. Some studies have suggested it might be related to risk taking (i.e. bungie jumping off a bridge).
- Some have tied certain variants of the DRD4 gene to curiosity, restlessness and the urge to explore.

Appendix: DNA Testing of LDHA and DRD4

- In 2015, Proskura *et al* published a paper in the journal *Animal Genetics* which studied the association between nucleotide variations at various locations in the DRD4 gene and racing pigeon performance.
- At two of these locations (g.129954 and g.129456) differences in nucleotide sequences were found to be correlated with race performance for race distances of less than 400 km (249 miles).
- The precise mechanism by which the DRD4 gene influences pigeon racing performance is not understood at this time. Given the wide range of effects found in humans for mutations of this gene, this will most likely not be an easy question to answer.

Appendix: DNA Testing of LDHA and DRD4

- However, the suggestion that some mutations in humans might be related to risk taking intrigues me.
- When I consider the race performance of “The Freak” one explanation would be that once he had oriented, he was ready to start for home and didn't wait for the safety of a group to fly home with. He took the risk of flying home alone. Since he was correct in his orientation, he got a big jump on the rest of the birds and beat them all, along with me.
- This could also explain why some birds I have had were either near the top or near the bottom on many of their races. They might have been risk takers and when they were right about orientation, they won. When they were wrong, they headed off with full gusto in the wrong direction.

Appendix: DNA Testing of LDHA and DRD4

- Several of my friends and colleagues have said they think they lose a higher percentage of their DRD4-T young birds off the landing board during settling. Again, this makes sense if the mutant gene is associated with risk taking. These youngsters take the risk of flying off before they really know what they are doing.
- This suggests that when selecting for the desired DRD4 alleles, strong selection pressure for homing desire and ability should also be employed.

Appendix: DNA Testing of LDHA and DRD4

- Whatever the mechanism, the results of this paper show a correlation and we can use this to our advantage as animal breeders. As was the case with LDHA, additional research is clearly needed.
- The 2015 paper studied race performance for the nine possible genotypes they identified for the DRD4 gene in pigeons:

  CCCC, CCCT, CCTT, CTCC, CTCT, CTTT, TTCC, TTCT, TTTT
- Not all nine genotypes were found in the test population. Of those that were found, there was a statistically significant difference between the race points earned by the **CTCT** birds (68.95) verses the CCCC (29.08), CCCT (35.24), CTCC (30.63) and TTCC (29.24) birds.
- It is possible the TTTT, CTTT, TTCT and/or CCTT genotypes are also beneficial, but they are rare enough that none were found in the test group. Indeed, the TT allele may not exist.

Appendix: DNA Testing of LDHA and DRD4

Lets look in more detail at the DRD4 genotypes

- I mentioned earlier that there were two locations within the DRD4 gene where nucleotide differences were correlated with race performance. This is a very important point.
- The first location is identified as g.129954 and the second is identified as g.129456. The original scientific paper published by Proskura *et al* used a notation where the genotypes were represented with the g.129954 location first and the g.129456 location second. In other words, when Proskura *et al* referred to a CTCC genotype they meant that at the g.129954 location the bird had C in one gene and a T in the other gene and for location g.129456 both genes had a C. This location order (954 then 456) is the most widely used to this day.

Appendix: DNA Testing of LDHA and DRD4

Lets look in more detail at the DRD4 genotypes

- HOWEVER, Genecheck, Inc in the U.S. reports their results in the opposite order (though their report is clearly labeled with respect to location). Many people who use Gene Check do not understand this location order distinction and it has lead to some problems.
- For example, two breeders wanted to trade some birds so that they could both breed CTCT birds. One reported that he had some CTCC birds and the other said he had some CCCT birds. Unfortunately they were using different location orders and the breeders actually BOTH had CT(g.129954)CC(g.129456) birds. The trade would have been of no benefit to either breeder.
- ALWAYS be clear about the location order you are using when talking about DRD4 genotypes. For the rest of these slides I will use the subscript $_4$ for location g.129954 and the subscript $_6$ for the location g.129456 (i.e. $C_4C_4T_6T_6$).

Appendix: DNA Testing of LDHA and DRD4

Lets look in more detail at the DRD4 genotypes

- DRD4 gene is not located on the sex chromosome (Z) and so both cocks and hens have two copies of the gene, one each from each parent.
- To date, only three alleles for the DRD4 gene have been observed: C_4T_6, T_4C_6 and C_4C_6. The T_4T_6 allele is theoretically possible, but its theoretical frequency is very low. Until such time as it is actually found, it is best to ignore it.
- This means that there are only six possible genotypes for the DRD4 gene. These are shown on the next two slides.

Appendix: DNA Testing of LDHA and DRD4

The six possible DRD4 genotypes that can be produced from the three known DRD4 alleles:

- C_4C_6 allele (i.e. sperm) + C_4C_6 allele (i.e. egg) = $C_4C_4C_6C_6$
- C_4C_6 allele + C_4T_6 allele = $C_4C_4C_6T_6$
- C_4C_6 allele + T_4C_6 allele = $C_4T_4C_6C_6$
- C_4T_6 allele + T_4C_6 allele = $C_4T_4C_6T_6$
- C_4T_6 allele + C_4T_6 allele = $C_4C_4T_6T_6$
- T_4C_6 allele + T_4C_6 allele = $T_4T_4C_6C_6$

Note: The order of the C's and T's at a particular location is interchangeable
So, $C_4C_4C_6T_6$ represents the same as $C_4C_4T_6C_6$

Appendix: DNA Testing of LDHA and DRD4

Lets look at the possible offspring from a mating of two $C_4T_4C_6T_6$ birds:

Egg \ Sperm	C_4T_6	T_4C_6
C_4T_6	$C_4C_4T_6T_6$	$C_4T_4C_6T_6$
T_4C_6	$C_4T_4C_6T_6$	$T_4T_4C_6C_6$

That's interesting! $C_4T_4C_6T_6$ x $C_4T_4C_6T_6$ doesn't breed true (only 50% are $C_4T_4C_6T_6$)!

Is there a mating which will produce 100% $C_4T_4C_6T_6$?

Appendix: DNA Testing of LDHA and DRD4

Turns out there is! $T_4T_4C_6C_6$ x $C_4C_4T_6T_6$:

Bird 2 \ Bird 1	T_4C_6
C_4T_6	$C_4T_4C_6T_6$

So here is what I am doing:

- I am working to eventually get all my families to be 100% either AA $C_4C_4T_6T_6$ or AA $T_4T_4C_6C_6$.
- This will allow me to make my crosses for the races and 100% of the youngsters will be AA $C_4T_4C_4T_4$ and they will have 100% hybrid vigor.
- I say “eventually” because this must not be done at the expense of the other “98” genes! It will be done over time.

Appendix: DNA Testing of LDHA and DRD4

Two final points -

1) What would you do with a super performing bird that tested BB for LDHA and CCCC for DRD4 (the lowest performing genotypes) ?
   - **Stock it!** It obviously has the right alleles for many of the other important genes for which we do not yet have DNA tests available. Since we have DNA testing for LDHA and DRD4 these two will be relatively easy to add.
   - At this point it is harder to get the right alleles for the “98” other genes and so efforts on that front should NOT be slowed in an effort to get “A”s and “T”s.
2) At this time there are actually four genes that can be DNA tested in racing pigeons. The other two are related to feather quality (feather keratin or F-KER) and muscling (myostatin or MSTN). I don't test for these yet because the alleles have not been correlated to race performance.