Population and Genetic monitoring the *Puccinia triticina* to provide food safety of Russia

T M Kolomiets, A I Zhemchuzhina, M I Kiseleva and N S Zhemchuzhina

State Scientific Institution "All-Russian Research Institute of Phytopathology", Russian Agricultural Academy", st. Institute, 5, Bolshie Vyazemy, Odintsovo distr., Moscow reg., 143050, Russia.
Corresponding author’s e-mail: lomi1@yandex.ru

Abstract. For determine the virulence of *P. triticina* populations in 7 regions of Russia there were tested 564 isolates on 49 wheat lines possessed juvenile resistance to the pathogen. There were identified from 37 to 42 virulence genes in fungus populations from different regions. The similarities of *P. triticina* populations were found in the presence of virulence genes *p1, p2c, p3a, p3bg, p3ka, p10, p14a, p14b, p17, p18, p21, p27+31, p30, p32, p33, p39, p40, pB, p22a* and in the absence of genes *p24, p29, p41, p42, p45, p47, p51, p53*. The differences were recorded by the frequency of individual virulence genes in fungus populations, for example the genes *p2a, p29, p38* weren’t identified in Middle Volga region, *p22a* – in Low Volga, *p19, p28, p22a, p38* – in Volga-Vyatka, *p36, p44* - in West Siberian. Leaf rust resistance genes were classified into 5 groups according to the degree of effectiveness. The resistance genes: *Lr24, Lr29, Lr41, Lr42, Lr45, Lr47, Lr51*, and *Lr53* were assigned to the effective group, since the corresponding wheat lines were not affected by any of the studied *P. triticina* populations. Wheat lines or varieties with identical resistance genes can be recommended to breeders as sources of resistance against leaf rust.

Key words: *Puccinia triticina*, population, monoisolates, virulence genes, monogenic lines, resistance, gene efficiency, wheat.

1. Introduction.
Wheat is the main food crop in Russia. According to the Ministry of agriculture of the Russian Federation, it occupies more than 85% of all areas under grain crops. In the total volume of grain production, it accounts for more than 60%. The yield of wheat in Russia is 25-27 cent/ha, while in the European Union this figure is 55 cent/ha, and the maximum yield in the world reaches 98 cent/ha.

High potential yield is often unrealized due to the effect of phytopathogens. Leaf rust (*Puccinia triticina* Eriks.) is one of the most common and harmful wheat diseases not only in Russia, but also in Western and Eastern Europe, North and South America, Asia, Australia and Africa [1-4]. Intensive development of the fungus in different climatic zones is due to the plasticity and high reproductive and migration capacity of the pathogen [5, 6]. In India, leaf rust is one of the most harmful diseases on...
wheat. The last epiphytotics in this country were observed in 2009-2011. [7]. In the United States and Canada, leaf rust epiphytoties occur once every 3 years, and crop losses can reach 50% [8]. In the United States at the beginning of the XXI century, new races virulent to the resistance gene *Lr17* appeared. They were identified in winter wheat varieties cultivated in the Great Valley. There were held epiphytoties in Texas, Oklahoma, Kansas, and Nebraska with the appearance of new races possessed *p17* virulent to winter wheat varieties there. Then these races spread to spring and soft winter wheat in the Eastern regions and California. As a result, the accumulation of the *p17* gene in the population, a complementary resistance gene in wheat varieties, led to significant crop losses in 2008, 2009 and 2010 in Argentina and Chile [9, 10]. In connection with this fact, the FAO has created a program to protect wheat from rust for developing countries in Africa, Central and East Asia, where wheat is the most important food crop[11, 12].

Leaf rust annually takes up to 10% of the grain harvest, and in the years of epiphytotic development, yield losses can reach more than 40% in Russia. In the main areas of grain production (Central Chernozem, North Caucasian, Central and Volga regions), epiphytoties occur often - 3-4 times in 10 years [13]. In the area of the Middle Volga region, yield losses from leaf rust reach 30%, with irrigation - 35%, and in years of strong epiphytoties they reach 62% [14-16].

The main reasons for the spread and harmfulness of leaf rust in Russia are the genetic uniformity of cultivated varieties and uncontrolled use of large areas of wheat varieties with race-specific resistance. The share of closely related varieties in the areas of wheat cultivation in Russia exceeds 50%. Due to the genetic similarity of cultivated wheat varieties, the gene pool of local varieties adapted to adverse biotic and abiotic factors changes and becomes impoverished. All this determines the susceptibility of wheat to the pathogen [17, 18].

Leaf rust populations are subject to qualitative and quantitative changes in composition over time and space. New pathotypes of the fungus can occur by mutations from avirulence to virulence, as a result of recombinative variability, or by increasing the frequency of a previously rarely encountered virulence gene, which increases the likelihood of its combination with other genes.

There are many examples of this. Thus, in the non-Chernozem areas with increasing acreage under the winter wheat variety Moscow 39 possessed the race-specific resistance gene *Lr 1*, there were observed the progress of the race frequency from 40% to 100% over several years [19]. The appearance of new spring wheat varieties of Omsk selection with the effective *Lr 9* gene on large areas in Western Siberia led to the identification of leaf rust races with the *p9* gene in 2007 by L. V. Meshkova and A. I. Zhemchuzhina in 2009 [20, 21].

The resistance gene *Lr 19* remained effective to leaf rust in varieties of domestic selection for more than 40 years. After breeders began to actively use this gene to create new varieties that were widely distributed in the Volga region and the Urals, rust races with the virulence gene *p19* were appeared. They were first discovered in 1997 in the Saratov region on wheat cultivars L 503 and L 505 [22]. Races possessed the *p19* gene quickly spread on wheat crops in the Central Chernozem, Central, Volga-Vyatka and other regions of the European part of Russia, and in 2000-2003 they were found in Western and Eastern Siberia and Far East territory [15].

In all these cases, the main reason for the loss of race-specific resistance to leaf rust by wheat varieties there was the increase in acreage under these varieties. As a result, the appearance of new aggressive races of leaf rust and mass cultivation of susceptible wheat varieties led to an increase in crop yield losses [15, 23, 24].

Annual fluctuations in the frequency of occurrence of prevailing races are the result of changes in weather conditions and cultivated wheat varieties, which have a significant effect on the selection of pathotypes adapted to them [25]. The main factor affecting the virulence genes dynamics is the resistance of cultivated varieties.

There were shown by the history of wheat breeding for leaf rust resistance, varieties possessed age and partial resistance provided longer protection to disease. The main criteria for selecting wheat varieties with long-term stability (including age-related) were the dynamics of the disease in the field, expressed by the area under the disease development curve (AUDPC), the latent period, the size of
pustules and their sporulating ability [24, 26, 27]. Analysis of literature data there has been shown that \( Lr \) lines with adult plant resistance genes may also possess race-specific resistance [26, 31]. Thus, a line with the \( Lr34 \) resistance gene in combination with other genes shows high field resistance [32, 33].

In this regard annual monitoring of the intraspecific structure of \( P. triticina \) populations makes it possible to track the frequency and dynamics of virulence genes in wheat crops. In addition, the study of the frequency of virulence genes was allowed us to determine the degree of effectiveness of known resistance genes and establish the prospects for their inclusion in the selection process to create new rust-resistant wheat varieties [28-30].

For 60 years in the Institute of Phytopathology there has been monitoring the virulence and racial compositions of \( P. triticina \) populations and identifying effective resistance genes on the territory of wheat cultivation in the Russian Federation. Information about the genetic structure of pathogen populations permit to select races and pathotypes of the fungus to create artificial infectious backgrounds, as well as to determine the genotype of resistance of wheat varieties to the pathogen leaf rust.

The aim of this work was to analyze the possibility of predicting the appearance of new potentially dangerous races and phenotypes of the fungus based on the study of the dynamics of virulence genes in \( P. triticina \) populations from various regions of Russia in 2009-2019.

2. Materials and methods

In 2009-2019 there were collected wheat samples with signs of leaf rust as a result of route surveys of wheat crops in the Central, Central Chernozem, North Caucasus, Volga-Vyatka, Middle Volga, Low Volga and West Siberian regions.

There were allocated 564 leaf rust isolates and produced on a susceptible variety (15-20 mg urediniospores of each isolate). They were stored in REVCO freezers at a temperature -80°C in the State Collection of Phytopathogenic Microorganisms. Immediately before determining the virulence genes, the isolates were removed from storage and removed from anabiosis using a special technique.

The virulence of mono-isolates was studied on a set of 42 \( Lr \) lines possessed juvenile resistance genes: \( Lr \, Lr \, 1, Lr \, 2a, Lr \, 2b, Lr \, 2c, Lr \, 3a, Lr \, 3b, Lr \, 3a, Lr \, 9, Lr \, 10, Lr \, 11, Lr \, 14a, Lr \, 14b, Lr \, 15, Lr \, 16, Lr \, 17, Lr \, 18, Lr \, 19, Lr \, 21, Lr \, 23, Lr \, 24, Lr \, 25, Lr \, 26, Lr \, 27 + Lr \, 31, Lr \, 28, Lr \, 29, Lr \, 30, Lr \, 32, Lr \, 33, Lr \, 36, Lr \, 38, Lr \, 39, Lr \, 40, Lr \, 41, Lr \, 42, Lr \, 44, Lr \, 45, Lr \, 46, Lr \, 47, Lr \, 51, Lr \, 53, LrB \), and on 7 lines possessed adult plant resistance genes: \( Lr \, 12, Lr \, 13, Lr \, 22a, Lr \, 22b, Lr \, 34, Lr \, 35, Lr \, 37 \) [34].

All work on isolation, reproduction of isolates, identification of virulence genes in them was carried out in the chambers of the artificial climate laboratory under optimal conditions: average daily air temperature +20°C, air humidity 60% during the day and 70% at night, illumination from 10,000 to 15,000 Lux, lighting period of 16 hours [35].

Identification of virulence genes to lines with juvenile resistance genes was performed during the germination phase on hydroponic culture [36]. Virulence genes for lines with age plant resistance genes were carried out in the flag-leaf phase in a greenhouse on a vase culture. The type of reaction of wheat \( Lr \) lines to fungus isolates was determined by the Mains and Jackson scale [37]. Scores of 3, 4, X, X+ indicate the susceptibility of the \( Lr \) line, i.e. the presence of a complementary virulence gene. Scores 0; 1, 2, X- indicate the stability of the \( Lr \) line, i.e., the absence of a complementary virulence gene.

The effectiveness degree of wheat resistance genes to leaf rust was revealed by virulence studying of isolates to monogenic wheat lines. According to the degree of effectiveness, resistance genes were divided into groups: effective (no more than 20% of isolates are affected from all tested), medium effective (from 21% to 50%), weakly effective (from 51% to 100%) [38, 39].

3. Finding and discussion

The study of virulence of 564 leaf rust isolates was given a permit us to identify virulence genes in \( P. triticina \) populations of 7 regions of the Russian Federation in 2009-2019.
There were identified 35 virulence genes in leaf rust populations from the North Caucasus and Low Volga regions, 34 genes from the Central region, 33 genes from the Central Chernozem, Middle Volga and West Siberian regions, and 31 virulence genes from the Volga-Vyatka region for set of lines possessed juvenile resistance genes. 

Genes \textit{p1}, \textit{p2c}, \textit{p3a}, \textit{p3bg}, \textit{p3ka}, \textit{p10}, \textit{p14a}, \textit{p14b}, \textit{p17}, \textit{p18}, \textit{p21}, \textit{p27+31}, \textit{p30}, \textit{p32}, \textit{p33}, \textit{p39}, \textit{p40}, \textit{p8}, \textit{p22b} were detected in \textit{P.triticina} populations with a high frequency (51-100%), and \textit{p24}, \textit{p29}, \textit{p41}, \textit{p42}, \textit{p45}, \textit{p47}, \textit{p51}, \textit{p53} were not detected. Populations of \textit{P.triticina} differed mainly in the number and frequency of virulence genes.

In regions where studies were conducted for at least 2 years, the dynamics of virulence genes was tracked. Thus, during the research period, positive dynamics was observed in \textit{P.triticina} populations from the Central region and the North Caucasus, i.e. an increase in the frequency of virulence genes \textit{p2a}, \textit{p15}, \textit{p25}, and in the population from the Volga-Vyatka region - the \textit{p16} gene. Negative dynamics, i.e. a decrease in the frequency of virulence genes was recorded in the fungus populations in the Volga-Vyatka region for the \textit{p2a} gene, in the North Caucasus - \textit{p11}, in the Central region and the North Caucasus - \textit{p16} and \textit{p23}.

The frequency of \textit{p9} and \textit{p38} genes increased significantly in the Central region and the North Caucasus, while the \textit{p19} gene decreased in the Central region, but increased in the North Caucasus.

It should be noted that in the population of \textit{P. triticina} from the Central region, the frequency of occurrence of virulence genes \textit{pp 1, 2c, 3a, 3bg,3ka, 10, 14a, 14b, 17, 18, 21, 25, 27+31, 30, 32, 33, 39, 40, B}, remained consistently high annually (75-100%). Some virulence genes so as \textit{24, 29, 36, 41, 42, 45, 47, 51, 53}, were absent in \textit{P. triticina} populations or met with a low frequency (from 2 to 16%). Frequencies of occurrence in the average years for gene \textit{p26} and \textit{p19} were at the level of 50% and 20% accordingly.

The frequency of occurrence of 13 virulence genes \textit{pp 2a, 2b, 9, 11, 15, 16, 20, 23, 28, 38, 44, 46}, varied significantly over the years. High content of genes \textit{pp 2a, 9, 15, 38 (76-100%)} in \textit{P. triticina} population in 2013, and their decline in subsequent years, up to the elimination of the \textit{p9} and \textit{p38} genes in 2016 – 2018 were noted.

In 2013 \textit{P.triticina} population from the Moscow region was missing the \textit{p16} gene, but \textit{p11} and \textit{p46} were detected with a moderate frequency (33 -38%). When analyzing the frequency of occurrence of these genes in populations in 2014, 2015, 2016, 2018 and 2019, their growth was noted up to 80-100%.

In \textit{P. triticina} population from the Central Chernozem region, 33 virulence genes were identified that overcome the resistance of juvenile resistance lines. Genes \textit{pp24, 29, 38, 41, 42, 45, 47, 51, 53} were absolutely effective, \textit{pp9, 46} - effective, \textit{pp2a, 15, 19, 28, 36, 39, 40, 44} – medium-effective juvenile resistance genes.

Populations of \textit{P. triticina}, represented by isolates from the Krasnodar and Stavropol territories (North Caucasus region), were similar in virulence structure, but had some differences. Thus, the frequency of occurrence of \textit{p9} and \textit{p38} in the Stavropol territory was 2 times higher than in the Krasnodar region (78% and 44%, 56% and 29%, respectively), and \textit{p23} – on the contrary (44% and 73%).

Single isolates virulent to \textit{Lr 28} (Krasnodar territory) and to \textit{Lr 44} (Stavropol kr.) were found in these populations,

\textit{P. triticina} population from Middle Volga region was distinguished by the absence of the \textit{p2a} and \textit{p38} genes and the highest incidence of \textit{p44} (43%).

The dynamics of the frequency of occurrence of virulence genes \textit{p25}, \textit{p20}, \textit{p36} and \textit{p46} was contrasting in the Low Volga region. In the \textit{P. triticina} population there was noted the progress of the concentration of the \textit{p25} gene (from 0% to 77%) but the concentration of \textit{p20} (from 71% to 35%), \textit{p36} (from 48% to 0%) and \textit{p46} (from 22% to 6%) were fall down.

In the Volga-Vyatka region, the frequency of occurrence of certain genes in \textit{P. triticina} population varied significantly over the years. Thus, the frequency of occurrence of \textit{p2a} and \textit{p20} decreased strongly from 72% (2009) and 100% (2010) up to 30% (2019). The frequencies of occurrence
following virulence genes: \( p11 \) (from 72% to 100%), \( p16 \) (from 0% to 70%), \( p26 \) (from 0% to 100%) and \( p36 \) (from 27% to 80%) were increased in the region over the years.

A high concentration of certain virulence genes was detected in leaf rust population from the West Siberian region. For example, the frequency of occurrence of \( p28 \) was 67%, which is 4-10 times higher than in populations of leaf rust in other areas.

Observations of the dynamics of virulence genes in \( P. triticina \) populations from various regions of the Russian Federation have shown that, since 2000, the frequency of occurrence of \( pp \) 3a, 3bg, 3ka, 10, 14a, 14b, 17, 18, 21, 30, 33, B remains high and is 100% in most cases [21, 29].

In recent years there has been noted the appearance of fungus isolates virulent to wheat varieties possessed \( Lr9 \). In 2007 for the first time \( p9 \) was detected by L. V. Meshkova [20] in \( P. triticina \) population in the Urals and Siberia, and in 2010 it was isolated from uredopustules from spring varieties Chernyava and Chernyava 13, received from the Omsk region to VNIIF. Later \( p9 \) was found in other regions, which is associated with the wide distribution of spring wheat varieties with \( Lr \) 9. In the Moscow region the widespread Moskovskaya 24 variety with \( Lr9 \) led to an increase in the proportion of isolates with this gene to 76% in 2013 [15].

Populations of \( P. triticina \) were diverse in the composition and number of genes virulent to lines possessed adult plant resistance genes. Virulence genes in leaf rust isolates of all populations were represented by 6-18 phenotypes, the total potential of which suggests that the genes of adult plant resistance to leaf rust are ineffective or poorly effective. The exception was the \( p22a \) gene, which showed avirulence to \( Lr22a \) in the population of the pathogen from the Volga-Vyatka region and weak virulence (no higher than 10%) in the fungus populations from North Caucasus and West Siberian regions. Low concentration of virulence genes \( p13 \) (12-20%) was also observed in most tested \( P. triticina \) populations and \( p35 \) (8-41%), \( P37 \) (8-35%) – in populations from the West Siberian and Volga regions.

The frequency of occurrence of virulence genes in the \( P. triticina \) population indicates the degree of effectiveness of resistance genes to the corresponding juvenile or adult plant resistance genes. According to the degree of effectiveness, resistance genes are conditionally divided into five groups, including absolutely effective, i.e. resistant to all isolates, and absolutely ineffective, i.e. susceptible to all isolates of the fungus. Among them, the following groups were identified:

* absolutely effective genes - \( Lr \) 24, \( Lr \) 29, \( Lr \) 41, \( Lr \) 42, \( Lr \) 45, \( Lr \) 47, \( Lr \) 51, \( Lr \) 53;
* effective - \( Lr \) 28, \( Lr \) 22a;
* moderately effective - \( Lr \) 13;
* weakly effective - \( Lr \) 1, \( Lr \) 3ka, \( Lr \) 10, \( Lr \) 14a, \( Lr \) 14b, \( Lr \) 15, \( Lr \) 20, \( Lr \) 27 + \( Lr \) 31, \( Lr \) 32, \( Lr \) 40, \( Lr \) 22b;
* completely ineffective - \( Lr \) 3a, \( Lr \) 3bg, \( Lr \) 17, \( Lr \) 18, \( Lr \) 30, \( Lr \) 33, \( Lr \) B (Table 1).

### Table 1. Effective resistance genes in various regions of Russia

| Region                  | Absolutely effective | Level of efficiency |
|-------------------------|----------------------|---------------------|
| Central                 | 24, 29, 36, 41, 42, 45, 47, 51, 53 | 9, 28, 38, 44 |
| Central Chernozem       | 9, 24, 29, 38, 41, 42, 45, 47, 51, 53 | 46 |
| Volgo-Vyatka            | 19, 24, 28, 29, 38, 41, 42, 45, 47, 51, 53 | 9, 25 |
| North Caucasus          | 24, 29, 41, 42, 45, 47, 51, 53 | 28, 44 |
| Low Volga               | 24, 29, 41, 42, 45, 47, 51, 53 | 9, 19, 28, 38, 44, 46 |
| Middle Volga            | 2a, 24, 29, 38, 41, 42, 45, 47, 51, 53 | 9, 16, 28, 36 |
| West Siberian           | 24, 29, 36, 41, 42, 44, 45, 47, 51, 53 | 23, 28, 38 |

The degree of effectiveness of the remaining \( Lr \) genes was ambiguous in the regions (Table 2).
The *Lr2* showed high efficiency in Middle Volga, average efficiency in Central region, weak efficiency in North Caucasus, in Volga-Vyatka and Low Volga regions, and was ineffective in Western Siberia.

The *Lr2b* and *Lr 2c* were moderately effective in Middle Volga, while they were ineffective in other regions.

The *Lr 9* was medium-effective in North Caucasus and Western Siberia, while it was ineffective in other regions.

The *Lr 11*, *Lr 21*, and *Lr 23* were medium-effective in Middle Volga, and ineffective in the other of the regions.

Table 2. Efficiency level of wheat resistance genes to *P. triticina* populations in various regions of the Russian Federation

| Region            | Moderately effective | Weakly effective | Absolutely effective |
|-------------------|----------------------|------------------|----------------------|
| Central           | 2a, 15, 19, 26       | 2b, 2c, 3ka, 16, 20, 23, 46 | 1, 3a, 3bg, 10, 11, 14a, 14b, 17, 18, 21, 25, 27+31, 30, 32, 33, 39, 40, B |
| Central Chernozem | 2a, 15, 19, 28, 36, 39, 40, 44 | 1, 2b, 2c, 3ka, 10, 14a, 14b, 16, 20, 21, 23, 25, 26, 27+31, 32 | 3a, 3bg, 11, 17, 18, 30, 33, B |
| Volgo-Vyatka      | 2a, 16, 26, 36, 44, 46 | 1, 2a, 2b, 2c, 3ka, 10, 11, 15, 20, 21, 23, 25, 26, 27+31, 32, 39, 40 | 3a, 3bg, 14a, 14b, 17, 18, 30, 33, B |
| North Caucasus    | 9, 16, 19, 36, 38, 46 | 1, 2a, 2b, 3ka, 10, 11, 15, 20, 21, 23, 25, 26, 27+31, 32 | 3a, 3bg, 14a, 14b, 17, 18, 30, 33, 40, B |
| Low Volga         | 16, 25, 26, 36       | 1, 2a, 2b, 2c, 3ka, 10, 11, 14a, 14b, 15, 20, 21, 23, 27+31, 32 | 3a, 3bg, 17, 18, 30, 33, 39, 40, B |
| Middle Volga      | 2b, 2c, 11, 19, 21, 23, 26, 44, 46 | 10, 15, 39, 40 | 1, 3a, 3bg, 3ka, 14a, 14b, 17, 18, 20, 25, 27+31, 30, 32, 33, B |
| West Siberian     | 16, 21, 26, 46       | 9, 14b, 15, 19, 32, 45 | 1, 2a, 2b, 2c, 3a, 3bg, 3ka, 10, 11, 14a, 17, 18, 20, 25, 27+31, 30, 33, 39, 40, B |

The *Lr 12* showed poor effectiveness in Western Siberia and moderate effectiveness in Central region, North Caucasus, and Middle Volga.

The *Lr13* was found to be moderate effective for all regions.

The *Lr16*, effective on Middle Volga and in Western Siberia, was moderately effective in other regions.

The *Lr19* was effective only in Low Volga region.

The *Lr 25*, effective in Volga-Vyatka region, showed moderate effectiveness in Low Volga and was ineffective in Central region, North Caucasus, and Middle Volga.

The *Lr 26* was weakly effective in North Caucasus and moderately effective in other regions.

The *Lr 36* was highly effective in Western Siberia, moderate – in Central, North Caucasus, and Low Volga regions, and weak in Volga – Vyatka region.

The *Lr 38* was moderate effective in North Caucasus and effective in Central, Low Volga, and West Siberian regions.
The *Lr44* is moderately effective in Volgo-Vyatka, Central Chernozem, and middle Volga regions, and has been shown to be effective in other regions.

The *Lr46* was effective in Low Volga region, but it was moderately effective in other regions.

The *Lr 34* was weakly effective in North Caucasus and Central region, but was moderately effective in the other of the regions.

The *Lr 35* was effective in Western Siberia and Low Volga was moderately effective in Central region and North Caucasus.

The *Lr 37* was weakly effective in Western Siberia, showed moderate effectiveness in other regions.

4. Conclusion

The degree of effectiveness of most of tested *Lr* genes was found to be weak to leaf rust in the territory of the surveyed regions of Russia.

Among the effective *Lr* genes, we should highlight the genes *Lr 24*, *Lr 29*, *Lr41*, *Lr 42*, *Lr 45*, *Lr 47*, *Lr 51*, *Lr 53*, which showed absolute effectiveness to leaf rust throughout the entire test period. Varieties with these resistance genes can be recommended to breeders as resistance donors to create new rust-resistant wheat varieties.

The resistance genes *Lr 28* and *Lr 22a* should not be included in the selection process, since hybrids with these genes will contribute to the accumulation of pathogen strains in the fungus population that were virulent to varieties with identical resistance genes, as was already the case with the genes *Lr 19*, *Lr 9*, *Lr 1* [20, 40, 41].

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